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SCIENTIFIC OPINION

Scientific Opinion on application (EFSA-GMO-UK-2008-60) for placing on the market of genetically modified herbicide tolerant maize GA21 for food and feed uses, import, processing and cultivation under Regulation (EC) No 1829/2003 from Syngenta Seeds[1]

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

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ABSTRACT

This Scientific Opinion reports on an evaluation of a risk assessment for placing on the market of genetically modified maize GA21 for food and feed uses, import, processing and cultivation. Maize GA21 was developed through particle bombardment and contains a single insertion locus consisting of modified maize *epsps* (m*epsps*) gene, conferring tolerance to glyphosate-based herbicides. Bioinformatic analyses and levels of the mEPSPS protein were considered sufficient. The comparative analysis of compositional, agronomic and phenotypic characteristics indicated that maize GA21 is not different from the conventional counterpart and its composition fell within the range observed among non-GM maize varieties, except for the presence of the mEPSPS protein in maize GA21. The safety assessment of maize GA21 identified no concerns regarding potential toxicity and allergenicity. A feeding study with broiler chickens confirmed that maize GA21 is as nutritious as its conventional counterpart. The EFSA GMO Panel considers that maize GA21 is unlikely to raise additional environmental safety concerns compared to conventional maize, but that its cultivation management could result in environmental harm under certain conditions. The EFSA GMO Panel therefore recommends managing the use of glyphosate on maize GA21 within diversified cropping regimes that have similar or reduced environmental impacts compared with conventional maize cultivation. The EFSA GMO Panel recommends the deployment of case-specific monitoring to address (1) changes in botanical diversity within fields due to novel herbicide regimes, and (2) resistance evolution to glyphosate in weeds due to novel herbicide regimes. The EFSA GMO Panel agrees with the general surveillance plan of the applicant, but requests that its proposals to strengthen general surveillance are implemented. The EFSA GMO Panel concludes that the information available for maize GA21 addresses the scientific comments raised by Member States and that maize GA21, as described in this application, is as safe as its conventional counterpart and commercial maize varieties with respect to potential adverse effects on human and animal health. If subjected to appropriate management measures, the cultivation management of maize GA21 is unlikely to raise safety concerns for the environment.

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[1] On request from the Competent Authority of the United Kingdom for an application (EFSA-GMO-UK-2008-60) submitted by Syngenta Seeds, Question No EFSA-Q-2008-481, adopted on 30 November 2011.

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[3] Acknowledgement: The Panel wishes to thank the members of the Standing Working Groups on Molecular Characterisation, Food and Feed, and Environment on GMO applications, the external experts Thomas Frenzel and Boet Glandorf, as well as as the EFSA staff member Yann Devos for the preparatory work on this Scientific Opinion, and the EFSA staff members Yann Devos (ENV), Zoltán Divéki (MC) and Antonio Fernandez Dumont (FF) for the support provided to the development of this EFSA scientific output.

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KEY WORDS

GMO, maize (*Zea mays*), GA21, herbicide tolerance, m*epsps*, risk assessment, food and feed safety, environment, environmental safety, food and feed uses, import and processing, cultivation, Regulation (EC) No 1829/2003

SUMMARY

Following the submission of an application (Reference EFSA-GMO-UK-2008-60) 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 herbicide tolerant genetically modified (GM) maize GA21 (Unique Identifier MON-ØØØ21-9) for food and feed uses, import, processing and cultivation.

In delivering its Scientific Opinion, the EFSA GMO Panel considered the application EFSA-GMO-UK-2008-60, additional information supplied by the applicant, scientific comments submitted by Member States, the environmental risk assessment report of the Czech Competent Authority (CZ CA), and relevant scientific publications.

Maize GA21 expresses a modified version of 5-enolpyruvylshikimate-3-phosphate synthase (mEPSPS), which is derived from maize EPSPS, and renders maize GA21 tolerant to the herbicidal active substance glyphosate.

The EFSA GMO Panel evaluated maize GA21 with reference to its intended uses and appropriate principles described in its guidelines for the risk assessment of GM plants and derived food and feed, the environmental risk assessment of GM plants, the selection of comparators for the risk assessment of GM plants, and for the post-market environmental monitoring of GM plants. The scientific evaluation of the risk assessment included molecular characterisation of the inserted DNA and expression of target protein. An evaluation of the comparative analyses of composition, agronomic and phenotypic characteristics was undertaken, and the safety of the new protein, and the whole food/feed was evaluated with respect to potential toxicity, allergenicity and nutritional quality. An evaluation of environmental impacts and the post-market environmental monitoring plan was undertaken.

The molecular characterisation data establish that maize GA21 contains a single insertion locus consisting of six contiguous complete or truncated versions of the m*epsps* expression cassette used for the transformation. No other parts of the plasmid are present in the transformed plant. Bioinformatic analyses of the open reading frames spanning the junction sites within the insert or between the insert and genomic DNA did not indicate specific hazards. The stability of the inserted DNA and the herbicide tolerance trait were confirmed over several generations. Updated analyses of the levels of mEPSPS in various plant parts collected from field trials performed in Europe were considered sufficient.

Based on the results of comparative analysis, the EFSA GMO Panel concludes that maize GA21 is compositionally, phenotypically and agronomically not different from the conventional counterpart, except for the presence of the mEPSPS protein in maize GA21. Bioinformatics studies demonstrated that the newly expressed mEPSPS protein shows no homology to known toxic proteins or allergens. The *in vitro* digestibility studies showed that most of the protein was degraded. There were no indications of adverse effects after administration of grain from maize GA21 to rats in a repeated-dose 90-day oral toxicity study. A feeding study with broiler chickens confirmed that grain from maize GA21 is as nutritious as grain of the conventional counterpart and a reference maize variety. Based on the available information, the EFSA GMO Panel is of the opinion that maize GA21 is as safe and nutritious as the conventional counterpart and reference maize varieties, and that it is unlikely that the overall allergenicity of the whole plant is changed.

Since the scope of the current application covers cultivation, the environmental risk assessment considered the environmental impact of full-scale commercialisation of maize GA21.

The CZ CA (including its Biosafety Commission) provided to EFSA its report on the environmental risk assessment of maize GA21 (dated 20 October 2010) on 25 October 2010 in line with Articles 6.3(c) and 18.3(c) of Regulation (EC) No 1829/2003. The report on the environmental risk assessment

of the CZ CA is provided in Annex H of the EFSA Overall Opinion, and has been considered throughout this EFSA GMO Panel Scientific Opinion.

The EFSA GMO Panel considers that maize GA21 has no altered agronomic and phenotypic characteristics, except for the herbicide tolerance. The likelihood of unintended environmental effects due to the establishment, survival and spread of maize GA21 is considered to be extremely low, and will be no different from that of conventional maize varieties.

It is highly unlikely that the recombinant DNA will transfer and establish in the genome of bacteria in the environment or human and animal digestive tracts. In the rare but theoretically possible case of transfer of the m*epsps* gene from maize GA21 to soil bacteria, no novel property would be introduced into the soil bacterial community and thus no positive selective advantage that would not have been conferred by natural gene transfer between bacteria would be provided.

Based on the evidence provided by the applicant and relevant scientific literature on maize GA21, the EFSA GMO Panel concludes that there are no indications of adverse effects on non-target organisms due to unintended changes in maize GA21, and therefore considers *trait*-specific information appropriate to assess whether maize GA21 poses a risk to non-target organisms.

The studies, supplied or reviewed by the applicant, showed no adverse effects on different types of non-target organisms due to the expression of the mEPSPS protein in glyphosate tolerant crops.

The EFSA GMO Panel does not expect potential adverse effects on biogeochemical processes and the abiotic environment due to the expression of the mEPSPS protein in maize GA21.

The EFSA GMO Panel is of the opinion that potential adverse environmental effects of the cultivation of maize GA21 are associated with the use of the complementary glyphosate-based herbicide regimes. These potential adverse environmental effects comprise (1) a reduction in farmland biodiversity, (2) changes in botanical diversity due to weed shifts, with the selection of weed communities mostly composed of tolerant species, and (3) the selection of glyphosate resistant weeds. The potential harmful effects could occur at the level of arable weeds, farmland biodiversity, food webs and the ecological functions they provide. The magnitude of these potential adverse environmental effects will depend upon a series of factors, including the specific herbicide and cultivation management applied at the farm level, the crop rotation and the characteristics of receiving environments.

The EFSA GMO Panel considers that the use of glyphosate-based herbicides at recommended field application rates of glyphosate on maize GA21 is unlikely to cause adverse effects to soil microbial communities or beneficial functions mediated by them.

The conclusions of the EFSA GMO Panel on the environmental safety of maize GA21 are consistent with those of the CZ CA. The CZ CA concluded that "*based on the existing information and data provided by the Syngenta Company within the evaluation process, the Czech CA considers that maize GA21 has no altered survival, multiplication or dissemination characteristics and interacts with other organisms as any conventional maize. However, the data presented on the issue of "Impacts of the specific cultivation, management and harvesting techniques" do not allow a comprehensive assessment of potential long-term effects on the environment associated to the use of the herbicide*" (section 9 of the environmental risk assessment report of the CZ CA). Hence, the CZ CA identified "*no potential effects on the environment either immediate, delayed, direct or indirect with the exception of those related to the change in the herbicide management*" (section 8.1 of the environmental risk assessment report of the CZ CA).

The EFSA GMO Panel anticipates that the repeated use of glyphosate at recommended application rates on continuous maize GA21 and/or other glyphosate tolerant crops grown in rotation may result in reductions in botanical diversity and/or weed density in maize fields to a level that might adversely affect food chains and webs, but not necessarily biological control functions, at the field and landscape level. Such a reduction in biodiversity may be considered problematic by risk managers depending upon protection goals pertaining to their region, especially in receiving environments that sustain little farmland biodiversity or in environmentally sensitive areas. Under such situations, the EFSA GMO Panel recommends that risk mitigation measures are put in place to manage potential herbicide effects, in order to ensure that glyphosate on maize GA21 is used within diversified cropping regimes that have similar or reduced adverse effects on farmland biodiversity compared with conventional maize cultivation. Possible risk mitigation measures include protecting adjacent habitats from herbicide drift, (re)introduction and better management of field margins or other 'out of crop' measures, less intense in-crop weed management, and especially rotating crops.

The cultivation of maize GA21 in monoculture or in rotation with other glyphosate tolerant crops, in conjunction with the repeated and/or exclusive application of glyphosate-based herbicides will cause changes in weed flora, and will favour the evolution and spread of glyphosate resistant weeds due to the selection pressure exerted by glyphosate. This, in turn, may affect food webs, and the functional value of weed vegetation for organisms of higher trophic levels (reduced functional biodiversity). Under such situations, the EFSA GMO Panel recommends that risk mitigation measures are put in place to delay resistance evolution. The selection pressure on weeds can be reduced by crop rotations (i.e., rotating glyphosate tolerant crops with non-glyphosate tolerant crops), using variable application rates and timing, applying a variety of herbicidal active substances with different modes of action, and by using non-herbicide weed control tools such as post-emergence cultivation and cover crops. To be most effective, these methods should be used in combination. A clear advantage of increasing cropping system diversification is that it would increase or conserve farmland biodiversity, as well as reducing the risk of weed shifts and the evolution of glyphosate resistant weed biotypes.

In its evaluation, the CZ CA was of the opinion that "*measures should be put in place under Directive 91/414/EEC and consecutively under Regulation (EC) No 1107/2009 to ensure compliance with regulatory requirements for the pesticide regimes used in Member States. These should include measures for the appropriate management of glyphosate on GMHT maize and for the development of weed resistance management strategies in each Member State permitting the use of glyphosate on maize GA21. It is necessary to exploit properly the appropriate antiresistance strategy to avoid undue herbicide usage*" (section 7.6.2 of the environmental risk assessment report of the CZ CA). The CZ CA recommended the applicant "*to design appropriately a relevant Technical User Guide for farmers that should involve Good Agricultural Practices of glyphosate applications guaranteeing sustainable and safe use of the entire GA21 technology*" (section 9 of the environmental risk assessment report of the CZ CA).

The EFSA GMO Panel gave its opinion and made recommendations on the scientific quality of the post-market environmental monitoring plan proposed by the applicant. In order to assess the efficacy of risk mitigation measures put in place to reduce levels of risk and in order to reduce the remaining scientific uncertainty identified in the environmental risk assessment, the EFSA GMO Panel recommends case-specific monitoring to address (1) changes in botanical diversity within fields due to novel herbicide regimes, and (2) resistance evolution to glyphosate in weeds due to novel herbicide regimes. The EFSA GMO Panel considers that risk managers should adapt monitoring methodologies to their local receiving environments, management systems and the interplay between the legislation for GMOs and plant protection products.

The EFSA GMO Panel agrees with the general surveillance plan for the cultivation of maize GA21 of the applicant (1) to establish farmer questionnaires as a reporting format of any on-farm observations of effects associated with the cultivation of maize GA21, (2) to use existing monitoring networks which observe changes in biota and production practices from farm up to regional level to obtain data on environmental impacts in the landscape where maize GA21 is cultivated, (3) to review all new scientific, technical and other information pertaining to maize GA21, and (4) to develop stewardship programs for the introduction, marketing, management and stewardship of maize GA21, but requests that its proposals and those made by the CZ CA to strengthen general surveillance are implemented. The EFSA GMO Panel agrees with the reporting intervals and modalities proposed by the applicant.

The general surveillance plan for the import and processing of maize GA21 was previously evaluated by the EFSA GMO Panel.

The CZ CA considered that "*case-specific monitoring is the most appropriate way to monitor identified potential environmental effects that could result from changes in the technology. Therefore it is proposed that the applicant performs a case-specific monitoring of long-term effects with focus on non-target organisms, weed shifts, the development of herbicide resistance to glyphosate and microbial biodiversity*" (section 8.1 of the environmental risk assessment report of the CZ CA).

The CZ CA considered that "*the general surveillance plan should be updated*" and made specific proposals to strengthen general surveillance (section 8.2 of the environmental risk assessment report of the CZ CA).

In conclusion, the EFSA GMO Panel considers that the information available for maize GA21 addresses the scientific comments raised by Member States and that maize GA21, as described in this application, is as safe as its conventional counterpart and commercial maize varieties with respect to potential adverse effects on human and animal health, in the context of its intended uses. The EFSA GMO Panel also concludes that maize GA21 is unlikely to raise additional environmental safety concerns compared to conventional maize, but that its cultivation management could result in environmental harm under certain conditions. The EFSA GMO Panel therefore recommends managing the use of glyphosate on maize GA21 within diversified cropping regimes that have similar or reduced environmental impacts compared with conventional maize cultivation. The EFSA GMO Panel recommends the deployment of case-specific monitoring to address (1) changes in botanical diversity within fields due to novel herbicide regimes, and (2) resistance evolution to glyphosate in weeds due to novel herbicide regimes. If subjected to appropriate management measures, the cultivation management of maize GA21 is unlikely to raise safety concerns for the environment.

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BACKGROUND

On 16 July 2008, the European Food Safety Authority (EFSA) received from the Competent Authority of United Kingdom an application (Reference EFSA-GMO-UK-2008-60) for authorisation of the herbicide tolerant genetically modified (GM) maize GA21 (Unique Identifier MON-ØØØ21-9), submitted by Syngenta Seeds under Regulation (EC) No 1829/2003. The scope of this application covers food and feed uses, import, processing and cultivation of maize GA21 and all derived products. After receiving the application EFSA-GMO-UK-2008-60 and in accordance with Articles 5(2)(b) and 17(2)(b) of Regulation (EC) No 1829/2003, EFSA informed both Member States and the European Commission, and made the summary of the application 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 12 September 2008, EFSA received additional information requested under completeness check (requested on 26 August 2008). On 21 October 2008, EFSA declared the application as valid in accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003.

On 1 October 2008, following a call for expression of interest among Competent Authorities under Directive 2001/18/EC and in accordance with Articles 6.3(c) and 18.3(c) of Regulation (EC) No 1829/2003, EFSA requested the Czech Competent Authority (CZ CA) to evaluate the initial environmental risk assessment of application EFSA-GMO-UK-2008-60 for the placing on the market of maize GA21 for cultivation. This call was initiated by EFSA on 1 August 2008 and the CZ CA gave its conformity on 8 October 2008.

EFSA made the valid application available to Member States and the European Commission, and consulted nominated risk assessment bodies of Member States, including national Competent Authorities within the meaning of Directive 2001/18/EC following the requirements of Articles 6(4) and 18(4) of Regulation (EC) No 1829/2003, to request their scientific opinion. Member States had three months after the date of acknowledgement of the valid application (21 January 2009) within which to make their opinion known.

The CZ CA asked the applicant for additional information on maize GA21 on 3 February 2009, 11 September 2009, and on 7 June 2010. The applicant provided the requested information on 13 July 2009, 27 October 2009, and on 21 July 2010, respectively.

The CZ CA (including its Biosafety Commission) provided to EFSA its report on the environmental risk assessment of maize GA21 (dated 20 October 2010) on 25 October 2010 in line with Articles 6.3(c) and 18.3(c) of Regulation (EC) No 1829/2003.

The Scientific Panel on Genetically Modified Organisms of EFSA (EFSA GMO Panel) carried out an evaluation of the scientific risk assessment of the GM maize GA21 for food and feed uses, import, processing and cultivation 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 its guidelines for the risk assessment of GM plants and derived food and feed (EFSA, 2006a, 2011b), the environmental risk assessment of GM plants (EFSA, 2010e), the selection of comparators for the risk assessment of GM plants (EFSA, 2011a), and for the post-market environmental monitoring of GM plants (EFSA, 2006b, 2011c); the scientific comments of Member States; the additional information provided by the applicant; the environmental risk assessment report from the CZ CA; and relevant scientific publications.

The EFSA GMO Panel asked the applicant for additional information on maize GA21 on 12 January 2010, 26 October 2010, 18 April 2011 and 3 May 2011. The applicant provided the requested information on 17 February 2010, 3 January 2011, 30 May 2011 and 4 October 2011. Additional information was also spontaneously provided by the applicant on 26 January 2010, 29 April 2011, 16 November 2011 and 28 November 2011. After receipt and assessment of the full data package, the EFSA GMO Panel finalised its risk assessment evaluation of maize GA21.

In giving its Scientific Opinion on maize GA21 to the European Commission, Member States and the applicant, and in accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003, EFSA has endeavoured to respect a time limit of six months from the acknowledgement of the valid application. As additional information was requested by both the CZ CA and 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).

The safety of the food and feed uses, import and processing of maize GA21 itself or as a component of stacked maize events has been evaluated previously by the EFSA GMO Panel under Regulation (EC) 1829/2003 (EFSA, 2007, 2009b, 2010a,b,c). The Commission Decision 2008/280/EC authorised the placing on the market of products containing, consisting of, or produced from maize GA21 pursuant to Regulation (EC) No 1829/2003 (EC, 2008a). Previously, the use of food and food ingredients produced from maize GA21 has been assessed (SCF, 2002) and approved under Regulation (EC) No 258/97 by the Commission Decision 2006/69/EC (EC, 2006), whilst other commercial uses have been assessed under Directive 2001/18/EC (SCP, 2000).

TERMS OF REFERENCE

The EFSA GMO Panel was requested to carry out a scientific risk assessment of maize GA21 for food and feed uses, import, processing and cultivation 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 environmental monitoring requirements based on the outcome of the risk assessment and, in 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 of the Cartagena Protocol, nor on the proposals for labelling and methods of detection (including sampling and the identification of the specific transformation event in the food/feed and/or food/feed produced from it), which are matters related to risk management.

ASSESSMENT

1. Introduction

Maize GA21 was developed to provide tolerance to the herbicidal active substance glyphosate by the introduction of a gene coding for the modified enzyme 5-enolpyruvylshikimate-3-phosphate synthase (mEPSPS). Glyphosate is normally phytotoxic to a broad range of plants. Its mode of action is to bind to and competitively inhibit the EPSPS protein, which is the key enzyme in the shikimate pathway that leads to the biosynthesis of the aromatic amino acids tyrosine, tryptophan and phenylalanine (Alibhai and Stallings, 2001; Dill, 2005; Duke and Powles, 2008b). The disruption of this pathway and the resulting inability to produce key amino acids prevents growth and ultimately leads to plant death. However, in case of maize GA21, a gene has been introduced that codes for the expression of the mEPSPS protein, which is insensitive towards inhibition by glyphosate. This protein is similar to the native EPSPS in maize, but it is not inhibited by glyphosate thus allowing the crop to be protected from the recommended dosages of glyphosate (Green, 2009; Dill et al., 2010)[4].

Maize GA21 was assessed with reference to its intended uses and the appropriate principles described in the EFSA GMO Panel guidelines for the risk assessment of GM plants and derived food and feed (EFSA, 2006a, 2011b), the environmental risk assessment of GM plants (EFSA, 2010e), the selection of comparators for the risk assessment of GM plants (EFSA, 2011a), and for the post-market environmental monitoring of GM plants (EFSA, 2006b, 2011c). In delivering its Scientific Opinion, the EFSA GMO Panel considered the information provided by the applicant in its application EFSA-GMO-UK-2008-60, and also (1) a review of peer-reviewed scientific data on maize GA21, (2) a report on areas and quantity of production, importation, use in Europe of maize GA21 and information on known and estimated human and animal exposure, (3) updated molecular characterisation, including sequence data for the flanking regions, (4) updated information on allergenicity and toxicology, (5) updated information on environmental issues, (6) post-market (environmental) monitoring plan, and (7) the additional information submitted by the applicant in reply to questions from both the EFSA GMO Panel and CZ CA.

The risk assessment evaluation presented here is also based on the scientific comments submitted by Member States (Annex G), the environmental risk assessment report of the CZ CA (Annex H), and relevant scientific publications.

2. Issues raised by Member States

The scientific comments raised by Member States are addressed in Annex G of the EFSA Overall Opinion[5], and have been considered throughout this EFSA GMO Panel Scientific Opinion.

3. Molecular characterisation

3.1. Evaluation of relevant scientific data

Unless specifically indicated, the information provided in this application, which is described in the following sections, has been evaluated previously by the EFSA GMO Panel (EFSA, 2007).

3.1.1. Transformation process and vector constructs[6]

Maize GA21 was developed to express a modified maize EPSPS protein to provide tolerance to glyphosate-based herbicides. Maize GA21 was produced by particle bombardment using a purified 3.49 kb *Not*I restriction fragment from the plasmid pDPG434. The fragment contains the m*epsps* expression cassette, consisting of a rice *actin*1 promoter (also including the first non-coding exon and intron), an optimised chloroplast transit peptide containing sequences from maize and sunflower, a modified maize *epsps* coding sequence and the 3' *nos* terminator from *Agrobacterium tumefaciens*

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[4] Technical dossier / Section D.1 / Page 17 / Appendix 4

[5] http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2008-481

[6] Technical dossier / Sections C1, C2, C3 and D1

(renamed as *Rhizobium radiobacter*). The modifications in the coding sequence of the m*epsps* gene led to amino acid changes at positions 102 (threonine to isoleucine) and 106 (proline to serine). As a result of these modifications, the expressed mEPSPS protein renders maize GA21 tolerant to glyphosate-based herbicides.

3.1.2. Transgenic constructs in maize GA21[7]

Molecular characterisation data demonstrated a single insertion locus in maize GA21 encompassing six contiguous complete or truncated copies of the *Not*I restriction fragment. The absence of vector backbone sequences in maize GA21 plants has been demonstrated by Southern analysis using a probe specific for the pDPG434 vector backbone (EFSA, 2007).

The sequences of the plant genome adjacent to the 3' and 5' ends were determined. Analyses of the flanking regions demonstrated that the 5' flanking sequence is of chloroplast origin and the 3' flank shows similarity to repetitive maize genomic sequences. Bioinformatic analysis of the 3' flanking sequence did not indicate insertion in a functional maize gene. Bioinformatic analysis did not show any biologically relevant similarity to allergens or toxins for any of the putative peptides that might be produced from open reading frames spanning the junctions of fragments within the insert or between the insert and genomic DNA (EFSA, 2007)[8].

Southern analysis of maize GA21 and maintenance of the phenotype indicated genetic and phenotypic stability of the event over multiple generations (EFSA, 2007).

3.1.3. Information on the expression of the insert[9]

The levels of mEPSPS protein in various parts of maize GA21 were analysed by enzyme-linked immunosorbent assay (ELISA).

Samples for analysis were collected from field trials conducted in the USA in 2004, 2005 and 2006 and in Europe (Spain) during 2007 and 2008. The analyses of the samples collected from various field trials performed in the USA were previously assessed by the EFSA GMO Panel (EFSA, 2007, 2009b, 2010a,b). For the European field trials, the following materials were tested: leaves and roots at whorl, anthesis and maturity stages; whole plants and pollen at anthesis stage; kernels at maturity stage. The plants were either treated or not treated with glyphosate.

The ranges of levels of the mEPSPS protein in various plant parts obtained from the European trials at the developmental stages where the expression was the highest are summarised in Table 1. The levels of the mEPSPS protein from field trials in Europe generally fell within the range of values previously reported for trials in the USA. Whilst there was variation in protein levels across the various field trials, such variation is not unexpected and is likely to result from differences in environmental conditions. Such variation in the levels of this non-toxic protein does not pose a safety issue. The results from Spain also indicate that the levels of the mEPSPS protein were not affected by treatment with glyphosate.

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[7] Technical dossier / Section D2

[8] Additional information received on 29 April 2011

[9] Technical dossier / Section D3

Table 1. Ranges in the levels of the mEPSPS protein in various maize GA21 plant parts (µg/g dry weight) in Spain (2007)

Plant parts	Developmental stage	mEPSPS
Leaves	Maturity	38.8-55.2
Roots	Anthesis	18.3-22.4
Whole plant	Anthesis	22.4-36.3
Pollen	Anthesis	121-143
Kernels	Maturity	6.85-9.65

3.2. Conclusion

The molecular characterisation data establish that maize GA21 contains a single insertion locus consisting of six contiguous complete or truncated versions of the m*epsps* expression cassette used for the transformation. Updated bioinformatic analyses of the open reading frames spanning the junction sites within the insert or between the insert and genomic DNA did not identify specific hazards. The stability of the inserted DNA and the herbicide tolerance were confirmed over several generations. The potential impacts of the mEPSPS protein levels, quantified in field trials carried out in Europe, are assessed in the food/feed and environment sections (see sections 5 and 6).

4. Comparative analysis

4.1. Evaluation of relevant scientific data

Unless specifically indicated, the information provided in this application, which is described in the following sections, has been evaluated previously by the EFSA GMO Panel (EFSA, 2007).

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

In the comparative analysis of maize GA21, which was previously evaluated by the EFSA GMO Panel (EFSA, 2007), maize GA21 was compared with near-isogenic non-GM controls. Whole crops and maize parts, including grains, were collected for compositional analysis from field trials. These field trials were performed during several seasons and at different locations (6 locations during 2 seasons in the USA (2004 and 2005); 5 locations in the USA (1996); 7 locations in the USA (1997); and 4 locations in Italy and Spain (1997)). In addition to the test maize and the near-isogenic non-GM controls, five or six commercial non-GM maize varieties were planted at each test site in 1997. The applicant justified the use of the respective non-GM control as the most appropriate comparator in each case. Maize GA21 treated with glyphosate as well as plants treated with conventional herbicides were included in the field trials. With respect to the comparative phenotypic and agronomic analysis, data were collected during field trials in the USA (1999 and 2004) and Brazil (2003).

In addition to these previously assessed studies, new field trials planted in Europe have been performed by the applicant for the comparative compositional analysis. Maize GA21 was compared with the conventional counterpart in six locations in Europe (Spain and Romania) in 2006. For additional comparative agronomic analyses, maize GA21 and the conventional counterpart were grown at eight locations in Europe (Spain, Romania and Czech Republic) in 2007, six locations in Europe (Spain and Romania) during the 2008 growing season[10] and at ten locations in the USA in 2005. Maize GA21 treated or untreated with glyphosate-based herbicide and the conventional counterpart were included (sections 4.1.2 and 4.1.3). The applicant justified the appropriateness of the

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[10] Additional information received 21 July 2010

comparators used by providing pedigree information[11] including breeding trees of the conventional counterpart and of maize GA21 grown in each field trial.

4.1.2. Compositional analysis[12]

In its previous Scientific Opinion on maize GA21 (EFSA, 2007), the EFSA GMO Panel has evaluated compositional data, which were obtained by analysis of materials from field trials performed during several seasons and at different locations (6 locations during 2 seasons in the USA (2004 and 2005); 5 locations in the USA (1996); 7 locations in the USA (1997); and 4 locations in Italy and Spain (1997)).

The set of compositional data from the field trials performed in the USA in 2004 and 2005 was used by the EFSA GMO Panel as the primary source for the comparative assessment of the composition of maize GA21. These data were analysed statistically both for each location and all locations combined, and the compounds analysed followed the recommendations of OECD (OECD, 2002). The EFSA GMO Panel was of the opinion that this dataset was in compliance with the principles described in its Guidance Document for the risk assessment of GM plants and derived food and feed (EFSA, 2006a, 2011b).

The data from proximate and mineral analyses of forage from maize GA21 (treated and non-treated with glyphosate) were compared to data for forage from the non-GM control and to the ranges of the analysed constituents in commercial maize varieties reported in the scientific literature (OECD 2002; ILSI, 2004). Statistically significant differences between maize GA21 and the non-GM maize control were observed for some parameters, i.e., lower overall levels of neutral detergent fibre and higher overall levels of phosphorous, but no differences were consistently observed over years and at each location (EFSA, 2007).

The composition of grain of maize GA21 and its non-GM control was analysed with respect to proximates, fatty acids, amino acids, minerals, vitamins and provitamins, anti-nutrients and other secondary metabolites (EFSA, 2007). The levels of β-carotene as well as cryptoxanthin, another carotenoid in the same metabolic pathway, were consistently statistically significantly higher, compared with those in the corresponding non-GM control. Additional information provided by the applicant upon request from the EFSA GMO Panel demonstrates that there are 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. Furthermore, all β-carotene and cryptoxanthin levels observed in grain 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 EFSA GMO Panel did not consider further compositional analysis of carotenoids necessary. In addition, the compositional analysis of grain from maize GA21 (glyphosate-treated and untreated) occasionally revealed statistically significant differences in the levels of some compounds compared to the non-GM control. However, none of these differences were consistently observed over years and at each location and the levels were within the normal ranges reported in the literature for commercial maize varieties (OECD, 2002; Reynolds et al., 2005).

The results of the compositional analyses of forage and grain from maize GA21, the corresponding non-GM comparator and commercial maize varieties obtained from the field trials performed in 1996 and 1997 are summarised by Sidhu et al. (2000). In these analyses, a more limited set of compounds was analysed, and only mean values and ranges calculated for the combined locations were provided. While some statistically significant compositional differences were detected, no consistent alterations were identified, and all levels fell within the ranges observed for commercial varieties as reported in the application or within ranges reported in the scientific literature (OECD, 2002). The EFSA GMO Panel concluded that the results do not indicate relevant compositional differences for forage and grain

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[11] Additional information received on 30 May 2011

[12] Technical dossier / Sections D7.1, D7.2 and D7.3

derived from maize GA21 compared with the corresponding non-GM comparator and the commercial varieties (EFSA, 2007).

The EFSA GMO Panel considered the observed compositional differences between maize GA21 and its non-GM comparators in light of the field trial design, the biological variation and the levels of the compounds in conventional maize, and concluded that the composition of grain and forage of maize GA21 falls within the normal ranges of conventional maize, except for the presence of the mEPSPS protein (EFSA, 2007).

Compositional data not previously assessed by the EFSA GMO Panel were obtained by analysis of forage and grain harvested from field trials performed in typical maize growing regions in Spain and Romania in 2006. Maize GA21 treated or untreated with glyphosate-based herbicide and the conventional counterpart were included in these field trials. Data on maize material from both individual and combined field trial sites were provided. Forage from maize GA21 and the conventional counterpart was analysed for proximates (moisture, protein, fat, ash, total carbohydrates), fibre (acid detergent fibre, neutral detergent fibre) and minerals (calcium and phosphorus). The compositional analysis of grain of maize GA21 and the conventional counterpart also included total dietary fibre, starch, fatty acids (palmitic, stearic, oleic, linoleic, and linolenic acid), amino acids (eighteen amino acids including aromatic amino acids), additional minerals (Co, Fe, Mg, Mn, K, Na, Se and Zn), vitamins (β-carotene, vitamin B1, vitamin B2, niacin, vitamin B6, pantothenic acid and vitamin E), anti-nutrients (phytic acid, raffinose, trypsin inhibitor) and other constituents (inositol, furfural, p-coumaric acid and ferulic acid). The selection of compounds followed the recommendations of OECD (2002).

The across location analysis of the composition of forage from maize GA21 and the conventional counterpart did not reveal statistically significant differences. A statistically significant difference was observed for total fat levels between maize GA21 treated with glyphosate-based herbicide and the conventional counterpart. This difference was small and not observed consistently at individual locations. Therefore, it was not considered biologically relevant by the EFSA GMO Panel. Mean values obtained for maize GA21 were all within the literature ranges reported for reference maize varieties.

The across location analysis of the composition of grain revealed statistically significant differences in some parameters, i.e. fiber fractions and vitamin E levels between the maize GA21 untreated with glyphosate-based herbicide and the conventional counterpart; and fiber fractions, ferulic acid and inositol between the maize GA21 treated with glyphosate-based herbicide and the conventional counterpart. However, these differences were not consistent at all individual locations. Several of the compounds analysed occurred at levels below the limit of quantification, i.e. selenium, sodium, raffinose and furfural.

For all plant constituents showing altered levels, the magnitude of the alteration were modest and the amounts detected were always within the ranges for levels reported in the scientific literature and in the ILSI Crop Composition Database (ILSI, 2006), with the exception of inositol. At three individual locations, inositol levels in maize GA21 were shown to be slightly above the range reported in the scientific literature. However, this result was only observed at one location in maize GA21 treated with glyphosate-based herbicide and two locations in maize GA21 untreated with glyphosate-based herbicide.

The EFSA GMO Panel requested additional information with regard to fiber components[13]. Statistically significant compositional differences in fiber contents between maize GA21 and the conventional counterpart were detected at one location; however, they were not observed consistently in any of the other seventeen sites evaluated.

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[13] Additional information received on 17 February 2010

The EFSA GMO Panel concludes that the compositional data obtained from field trials in Spain and Romania (2006) confirm the results of its previous evaluation (EFSA, 2007), and is of the opinion that maize GA21 is compositionally not different from the conventional counterpart and that its composition fell within the range observed among non-GM maize varieties, except for the newly expressed mEPSPS protein.

4.1.3. Agronomic traits and GM phenotype[14]

In its previous evaluation of maize GA21 (EFSA, 2007), the EFSA GMO Panel has considered extensive disease susceptibility and agronomic data (such as 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, which were collected during field trials over several seasons and at different locations (USA in 1999 and 2004; Brazil in 2003).

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, or across the two genetic backgrounds used in these studies, and all yield data fell within the range reported for non-GM maize varieties. In addition, differences in the number of emerged plants, plant height, and percent snapped plants were reported at some locations and were within the biological variation (ILSI, 2006). No differences that could indicate unintended effects of the genetic modification were found (EFSA, 2007).

The EFSA GMO Panel noted that in the course of the agronomic field trials conducted in Brazil in 2003, phytotoxicity was observed in up to 30 % of plants treated with glyphosate at one of the three sites. In the application, a high incidence of fungal disease in both maize GA21 and conventional maize was reported 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 EFSA GMO Panel accepted the explanation that the observed phytotoxicity resulted from the high incidence of fungi at this location (EFSA, 2007).

The EFSA GMO Panel concluded that the field trials did not reveal changes in phenotypic characteristics and agronomic performance, except for the introduced trait (EFSA, 2007).

Agronomic data not previously assessed by the EFSA GMO Panel were obtained during field trials at ten locations in the USA during the 2005 growing season, eight locations in Europe (Spain, Romania and Czech Republic) during the 2007 growing season and six locations in Europe (Spain and Romania) during the 2008 growing season[15]. The field trials performed in the USA included maize GA21 and the conventional counterpart untreated with the target herbicides. The European trials comprised additional plots of maize GA21 treated with the target herbicide. Extensive data on phenotypic characteristics, agronomic performance and disease susceptibility (e.g., grain yield, number of emerged plants, plant population at harvest, ear height, percent snapped plants, root lodging) were collected.

When analysed across all locations, statistically significant differences were observed for grain weight in the USA as well as in Europe during the 2007 growing season. In the European field trials in 2008, plant height, ear height, harvest population and grain yield were statistically significantly different between maize GA21 and the conventional counterpart. Some additional differences in agronomic data were detected at individual field trial sites only. The described differences are considered to be of small magnitude and not biologically relevant. No consistent trend was observed across locations and years.

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[14] Technical dossier / Section D7.4

[15] Additional information received 21 July 2010

The EFSA GMO Panel concludes that new agronomic data provided for USA and European locations confirm the previous assessment and is of the opinion that maize GA21 is agronomically and phenotypically not different from the conventional counterpart, except for the newly introduced trait.

4.2. Conclusion

Based on the results of comparative analyses, the EFSA GMO Panel concludes that maize GA21 is compositionally, phenotypically and agronomically not different from the conventional counterpart and its composition fell within the range observed among non-GM maize varieties, except for the presence of the mEPSPS protein in maize GA21.

5. Food/Feed safety assessment

5.1. Evaluation of relevant scientific data

Unless specifically indicated, the information provided in this application, which is described in the following sections, has been evaluated previously by the EFSA GMO Panel (EFSA, 2007).

5.1.1. Product description and intended use[16]

The scope of application EFSA-GMO-UK-2008-60 is for food and feed uses, import and processing as well as cultivation of maize GA21 in the EU.

The genetic modification results in the expression of the mEPSPS protein in maize GA21, which confers to the plants tolerance to the herbicidal active substance glyphosate. Thus, the modification is intended to improve agronomic performance only and is not intended to influence the nutritional properties, the processing characteristics and overall use of maize as a crop. Maize GA21 is intended to be processed like any conventional maize. The primary use of maize is for animal feed, but it is also processed into valuable food products such as starch, syrups and oils.

5.1.2. Effect of processing[17]

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, such as fibre, starch and germ meal (limit of detection of 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 the mEPSPS protein (limit of detection 0.02 µg mEPSPS/g sample).

The influence of temperature on the mEPSPS protein derived from a recombinant *Escherichia coli* strain (section 5.1.3.1) 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 this information and the data obtained in the comparative compositional analysis of raw agricultural commodities of maize GA21 and the conventional counterpart (section 4.1.2), the EFSA GMO Panel considers that there are no reasons to assume that the effect of processing on products derived from maize GA21 would be different from that on products from conventional maize.

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[16] Technical dossier / Section D7.5

[17] Technical dossier / Section D7.6

5.1.3. Toxicology[18]

5.1.3.1. Protein used for safety assessment

Due to the comparatively low expression level of the mEPSPS protein in maize GA21 and the difficult task to isolate a sufficient quantity of purified protein from the GM maize plant, the safety studies with the newly expressed protein were conducted with a mEPSPS protein produced in a recombinant *E. coli* strain. Maize GA21 contains both the newly expressed mEPSPS and the endogenous EPSPS protein. A comparison of the levels of the mEPSPS protein in leaves of maize GA21 with the levels of the endogenous EPSPS protein in leaves of non-GM maize (determined by ELISA) showed that the percentage of the mEPSPS protein was approximately 96 % of the total EPSPS protein in leaves of maize GA21. The structural and functional equivalence of the mEPSPS protein produced in *E. coli* to the protein expressed in leaves of maize GA21 was shown by N-terminal amino acid sequence analysis, SDS PAGE followed by Western analysis, MALDI-TOF mass spectrometry and an enzyme activity assay. In a study on protein glycosylation using a commercial glycan detection kit after SDS PAGE, neither the plant nor the microbial mEPSPS protein was found to be glycosylated. The EFSA GMO Panel accepted the *E. coli* derived mEPSPS protein as an appropriate substitute test material for the plant mEPEPS protein in the safety studies (EFSA, 2007).

5.1.3.2. Toxicological assessment of expressed novel proteins in maize GA21

EPSPS proteins occur in 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.

The mEPSPS protein expressed in maize GA21 is a modified version of the endogenous maize EPSPS protein. The amino acid sequence of the mEPSPS protein differs from the endogenous maize protein in two of a total of 445 amino acids. Threonine in position 102 of the EPSPS protein has been replaced by isoleucine in the mEPSPS protein, and proline in position 106 by serine, resulting in tolerance of the plants to glyphosate. Based on the DNA sequence information of the m*epsps* gene 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 protein predominantly expressed in maize GA21.

Bioinformatic analysis

A bioinformatics study, which was previously evaluated by the EFSA GMO Panel, revealed no relevant homology between the mEPSPS protein and known toxic proteins (EFSA, 2007).

In this application, a new study was provided[19]. Analysis of the amino acid sequence of the mEPSPS protein using the BLASTP search programme and an updated database (National Centre for Biotechnology Information [NCBI] Entrez Protein Database [NCBI, 2011]) revealed no relevant homology between the mEPSPS protein and known toxic proteins and thus confirmed the results of the previous study.

Degradation in simulated digestive fluids

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 gastric fluid (SGF)[20]. No intact protein (approximately 47.4 kDa) was detectable after incubation in SGF for one minute when the samples were analysed using SDS PAGE and protein staining. The EFSA GMO Panel noted that after incubation of the microbially produced mEPSPS protein in SGF for up to 60 minutes, diffusely stained

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[18] Technical dossier / Section D7.8
[19] Additional information received on 29 April 2011
[20] Technical dossier / Section D7.8.1 / Appendix 19

regions (approximately 4-5 kDa) were visible. However, 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 one minute. In the sample of plant-derived mEPSPS protein incubated for one minute, an immunoreactive fragment (approximately 6 kDa) was detected. The EFSA GMO Panel did not identify a safety concern regarding the potential presence of the fragment (EFSA, 2007).

Acute toxicity testing

There were no adverse effects in an acute oral toxicity study after administration of a single dose of 2000 mg mEPSPS/kg body weight to mice (EFSA, 2007)[21].

The EFSA GMO Panel considers that acute toxicity testing of the newly expressed proteins is of little additional value for the risk assessment of the repeated human and animal consumption of food and feed derived from GM plants.

5.1.3.3. Toxicological assessment of new constituents other than proteins

No new constituent other than the mEPSPS protein is expressed in maize GA21 and no biologically relevant changes in the composition of maize GA21 were detected in the comparative compositional analysis (section 4.1.2). Therefore, a toxicological assessment of new constituents is not applicable.

5.1.3.4. Toxicological assessment of the whole GM food/feed[22]

In a repeated-dose 90-day oral toxicity study, grain of maize GA21 was fed to Wistar-derived (Alpk:AP$_f$SD) rats as a component of the diet[23]. Groups of 12 male and 12 female animals were fed diets containing 10 % or 41.5 % (w/w) grain from maize GA21 sprayed with glyphosate (treated), 10 % or 41.5 % grain from maize GA21 sprayed with other herbicides (untreated), or 10 % or 41.5 % grain from the conventional counterpart 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, there were no biologically relevant differences between groups. Eye 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 % grain from maize GA21 treated with glyphosate showed a lower bodyweight compared with the respective control group in weeks 6, 10, 12, 13 and 14. These differences were not observed in males receiving diets with 41.5 % grain from untreated maize GA21. Furthermore, the group fed 41.5 % grain from the conventional counterpart showed a higher bodyweight gain in relation to all other test and control groups included in the study. In the absence of indications of adverse effects (see below), the EFSA GMO Panel does not consider the differences in bodyweight as toxicologically relevant (see also EFSA, 2007).

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); and 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

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[21] Technical dossier / Section D7.8.1 / Appendix 21

[22] Technical dossier / Section D7.8.4

[23] Technical dossier / Section D7.8.4 / Appendix 25

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. Furthermore, the findings were not accompanied by histopathological changes in the respective organs or tissues. Therefore, the EFSA GMO Panel does not consider the observed statistically significant differences as toxicologically relevant (see also EFSA, 2007).

In conclusion, there are no indications of adverse effects after administration of grain from maize GA21 to rats for 90 days.

5.1.4. Allergenicity[24]

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 (CAC, 2003; EFSA, 2006a, 2010g, 2011b).

5.1.4.1. Assessment of allergenicity of the newly expressed proteins

The *epsps* gene encoding the unmodified maize EPSPS protein was derived from maize, a source which is not regarded as commonly allergenic (section 5.1.4.2).

Bioinformatics-supported comparisons of the amino acid sequence of the mEPSPS protein with those of known allergens that were previously assessed by the EFSA GMO Panel, revealed no biologically relevant homology of the mEPSPS protein to known allergens (EFSA, 2007). A new bioinformatics study[25] provided by the applicant using an updated database (Food Allergy Research and Resource Program (FARP) AllergenOnline database, version 11.0) confirmed the results of the previous study.

The studies on degradation of the mEPSPS protein in simulated gastric fluid (section 5.1.3.2) 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 considered unlikely to raise concerns regarding allergenicity.

Based on this information, the EFSA GMO Panel considers it unlikely that the mEPSPS protein present in maize GA21 is an allergen.

5.1.4.2. Assessment of allergenicity of the whole GM plant

Rare cases of occupational allergy to maize dust have been reported in the scientific literature (Jeebhay and Quirce, 2007; Bardana, 2008). 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 individuals. In addition, most individuals with a positive skin prick test (SPT) or having IgE antibodies against maize were suffering from respiratory allergy and only a few ones displayed a true food allergy upon oral challenge with maize products (Jones et al., 1995; Pasini et al., 2002). Therefore, oral sensitisation to maize proteins is very rare.

The allergenicity of the whole crop could be increased due to an unintended change of the random insertion of the transgene in the genome of the recipient, for example through qualitative or

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[24] Technical dossier / Section D7.9

[25] Additional information received on 29 April 2011

quantitative modification of the pattern of expression of endogenous proteins. This issue does not appear to be a safety concern to the EFSA GMO Panel since maize is not considered a major allergenic food.

5.1.5. Nutritional assessment of GM food/feed[26]

A 49-day feeding study carried out with broiler chickens has been previously assessed by the EFSA GMO Panel (EFSA, 2007). Groups of 150 male and 150 female animals (Ross 344 males crossed with Ross 308 females) were fed with diets containing approximately 51-64 % (w/w) of maize grains depending on the growth status of the animals. The diets contained grains from maize GA21 treated with glyphosate, from maize GA21 treated with conventional herbicides, from the conventional counterpart treated with conventional herbicides or from a reference maize variety.

There were no adverse effects in this study. Although the diets were not completely identical with regard to nutrient composition, animals fed diets containing grain from maize GA21 showed no biologically relevant differences in mortality, body weight, feed conversion and carcass yield compared with animals receiving diets containing grain from the conventional counterpart and from a reference maize variety.

Thus, the broiler feeding study supports the results of the comparative compositional analysis and confirms that grain from maize GA21 is as nutritious as grain of the conventional counterpart and a reference maize variety.

5.1.6. Post-market monitoring of GM food/feed[27]

No biologically relevant compositional, agronomic and phenotypic changes were identified in maize GA21 as compared with the conventional counterpart, with the exception of the newly expressed protein. Furthermore, the overall intake or exposure is not expected to change due to the introduction of maize GA21 into the market. Therefore, and in line with its Guidance Document for the risk assessment of GM plants and derived food and feed (EFSA, 2006), the EFSA GMO Panel considers that post-market monitoring of the food/feed derived from maize GA21 is not necessary.

5.2. Conclusion

The mEPSPS protein expressed in maize GA21 differs from the EPSPS protein present in conventional maize in two amino acids. Bioinformatics studies demonstrated that the mEPSPS protein shows no homology to known toxic proteins and allergens. The *in vitro* digestibility studies showed that most of the protein was degraded. No toxicity of orally administered mEPSPS protein was observed in a study on acute toxicity using mice.

There were no indications of adverse effects in a repeated-dose 90-day rat oral toxicity study after administration of diets containing grain from maize GA21. In addition, a feeding study with broiler chickens confirmed that grain from maize GA21 is as nutritious as grain of the conventional counterpart and a reference maize variety. These studies therefore support the conclusions of the comparative analysis showing that maize GA21 is compositionally not different from the conventional counterpart, except for the presence of the mEPSPS protein. A review[28] of peer-reviewed scientific data on maize GA21 and derived food and feed, relevant for the safety assessment, revealed that there was no new information that would require changes of a previous EFSA GMO Panel Scientific Opinion on maize GA21.

The EFSA GMO Panel concludes that maize GA21 is as safe and as nutritious as the conventional counterpart and reference maize varieties and that it is unlikely that the overall allergenicity of the whole plant is changed.

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[26] Technical dossier / Section D7.10

[27] Technical dossier / Section D7.11

[28] Additional information received on 20 December 2010

6. Environmental risk assessment and risk management strategies

6.1. Evaluation of relevant scientific data

The scope of application EFSA-GMO-UK-2008-60 is for food and feed uses, import, processing and cultivation of maize GA21 and all derived products. Therefore, the environmental risk assessment is concerned with potential direct and indirect environmental effects of the cultivation and the spread of maize GA21 into non-cultivated environments, as well as indirect exposure through manure and faeces from animals fed maize GA21.

The EFSA GMO Panel considered the following issues in the environmental risk assessment submitted by the applicant (1) changes in plant fitness due to the genetic modification, (2) potential for gene transfer and its consequences, (3) interactions between the GM plant and target organisms, (4) interactions between the GM plant and non-target organisms, (5) effects on animal and human health, (6) interactions with biogeochemical processes and the abiotic environment, (7) impacts of the specific cultivation, management and harvesting techniques, and (8) risk management strategies (including post-market environmental monitoring).

The CZ CA provided to EFSA its report on the environmental risk assessment of maize GA21 (dated 20 October 2010) on 25 October 2010 in line with Articles 6.3(c) and 18.3(c) of Regulation (EC) No 1829/2003. The report on the environmental risk assessment of the CZ CA is provided in Annex H of the EFSA Overall Opinion, and has been considered throughout this EFSA GMO Panel Scientific Opinion.

6.2. Environmental risk assessment[29]

6.2.1. Changes in plant fitness due to the genetic modification[30]

A series of field trials with maize GA21 was conducted by the applicant at several representative maize growing locations in the USA during 2004 (8 locations) and 2005 (10 locations)[31] and in Europe in 2007 (3 sites in Czech Republic; 3 in Spain; 2 in Romania)[32] and 2008 (3 sites in Spain; 3 in Romania)[33] to compare the agronomic performance and field characteristics of maize GA21 with its comparators. Information on phenotypic and agronomic characteristics of maize GA21 and its comparators was generated to compare their growth habit, vegetative vigour and reproduction characters. Several endpoints related to growth habit, vegetative growth, reproduction, and yield and grain characteristics were measured[34].

A randomised complete block design with five and four replications per location was used in the USA field studies in 2004 and 2005, respectively, and maize GA21 and its comparators received the same conventional herbicide treatments. In the EU field studies, each of the agronomic trials was conducted using a randomised complete block design with four replications per location. In the 2007 EU field study, all plots were sprayed with conventional herbicides according to local practices. In addition to this, the "maize GA21 treated" plots were sprayed with glyphosate. In the 2008 EU field study, all plots in Spain were treated with a standard herbicide program according to local practices. In Romania, no conventional herbicides were used; only hand weeding was employed. In addition to this, the "maize GA21 treated" plots were sprayed with glyphosate in Spain and Romania[35].

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[29] Technical dossier / Section D9 / Appendix 28

[30] Technical dossier / Sections B2, B3, B4, D4, D9.1 and D9.2

[31] Technical dossier / Section D7.4 / Page 27 / Annexes 11 and 12: Meghji and Dunder (2005) & Beanblossom and Meghji (2007), respectively

[32] Technical dossier / Section D7.4 / Page 27 / Annex 13: Bérion (2008) // Additional information received on 16 November 2011

[33] Additional information received on 21 July 2010 / Question 2 / Page 5 / Appendix 2: Bérion (2010) / Appendix 3

[34] Additional information received on 13 July 2009 / Question 1 / Pages 5-6 / Appendix 1

[35] Additional information received on 16 November 2011

The breeding trees provided by the applicant confirmed that the near-isogenic lines used in the agronomic and phenotypic field trials had a comparable genetic background with maize GA21[36].

The agronomic and phenotypic field trial data did not show major changes in plant characteristics that indicate altered fitness, persistence and invasiveness of maize GA21 plants, though there is a potential for enhanced biomass production when glyphosate-based herbicides are applied. A number of endpoints (i.e., plant height, ear height, yield) showed statistically significant differences in the across-location comparisons between maize GA21 and its near-isogenic lines. These differences were not consistently observed in each location, and were not considered biologically meaningful with respect to persistence and invasiveness potential. Hence, the range of values for agronomic and phenotypic characteristics was shown to fall within the range of values observed for traditional maize hybrids. No visually observable response to naturally occurring insects, diseases and/or abiotic stressors recorded during the growing season provided any indication of altered stress responses of maize GA21 as compared with its conventional counterpart.

It is considered very unlikely that the establishment, spread and survival of maize GA21 would be increased due to the herbicide tolerance trait. This trait can only be regarded as providing a potential selective advantage to maize GA21 when glyphosate-based herbicides are applied. Moreover, it is considered very unlikely that maize GA21 plants or their progeny will differ from conventional maize varieties in their ability to survive as volunteers until subsequent seasons, or to establish feral populations under European environmental conditions (section 6.2.2.2). Maize is highly domesticated and generally unable to survive in the environment without management intervention (Baker, 1974; Bagavathiannan and Van Acker, 2008). The survival of maize is limited by a combination of low competitiveness, absence of a dormancy phase, and susceptibility to plant pathogens, herbivores and cold climatic conditions (van de Wiel et al., 2011). Maize plants are only winter hardy in European regions with mild winters, and in those situations maize kernels remaining in the field after harvest can germinate, grow, flower, and locally cross-pollinate neighbouring maize plants. The occurrence of maize volunteers was reported in Spain and other European regions (Gruber et al., 2008; Palaudelmàs et al., 2009), but these plants grow weakly and tend to flower asynchronously with the cultivated maize crops in which they occur (Palaudelmàs et al., 2009). While maize GA21 volunteers occurring in cultivated areas will be tolerant to glyphosate, they are normally controlled by current agricultural practices, including the use of selective herbicides and/or cultivation techniques (Beckie et al., 2006; Deen et al., 2006)[37]. If maize GA21 is rotated with broad leaf crops (such as soybean, oilseed rape, sugar beet, sunflower), potential volunteers can easily be controlled with selective graminicide herbicides. For the rotation of glyphosate tolerant maize and soybean, the control of maize volunteers in soybean has been achieved by the use of herbicide regimes involving a graminicide herbicide(s) and glyphosate-based herbicides in the USA (Deen et al., 2006). The EFSA GMO Panel notes that mechanical weed control such as hoeing is the only solution for weed control if maize GA21 is rotated with another maize crop (either conventional or tolerant to glyphosate), as effective herbicides cannot be applied without killing the rotational maize crop itself (Davis et al., 2008). Maize GA21 volunteers are likely to be controlled by the herbicide programmes applied in glufosinate-ammonium tolerant crops (Feng et al., 2010; Green and Castle, 2010; Green and Duke, 2011).

Despite cultivation for centuries, maize plants do not occur outside cultivated or in disturbed land in Europe. 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 GA21 and any change in survival (including over-wintering), persistence and invasiveness capacity. Because the general characteristics of maize GA21 are unchanged, herbicide tolerance is not likely to provide a selective advantage outside of cultivation in Europe.

Since maize GA21 has no altered agronomic and phenotypic characteristics, except for the herbicide tolerance, the EFSA GMO Panel is of the opinion that the likelihood of unintended environmental

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[36] Additional information received on 30 May 2011 / Annex 1

[37] Additional information received on 17 February 2010 / Question 5 / Pages 16-21

effects due to the establishment and survival of maize GA21 will be no different to that of conventional maize varieties.

The conclusion of the EFSA GMO Panel is consistent with that of the CZ CA. The CZ CA concluded that "*field trials with maize GA21 have not shown any increased invasiveness, weediness or changed fitness characteristics, except in the presence of glyphosate. All data support the conclusion that there are no biologically significant differences between Event GA21-derived maize hybrids and their corresponding non-transgenic near-isogenic hybrids, apart from the introduced trait of herbicide tolerance. Corresponding to the information presented by the applicant, the Czech CA 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*". "*The potential risk of the expression of the mEPSPS protein in Event GA21 maize resulting in a selective advantage or disadvantage can be considered negligible*" (sections 6.3.5 and 7.2 of the environmental risk assessment report of the CZ CA).

6.2.2. Gene transfer

The EFSA GMO Panel evaluated the potential for horizontal and vertical gene flow of maize GA21, as well as the potential environmental consequences of such 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 deoxyribonucleic acid (DNA), or vertical gene flow via the dispersal of pollen and seed.

6.2.2.1. Plant to bacteria gene transfer and its consequences[38]

Bacteria are capable of exchanging genetic material directly between each other and even across species boundaries using different mechanisms such as conjugation, transduction or natural transformation. DNA of plants, which may also include DNA derived from GM plants, could hypothetically be acquired by bacteria through horizontal gene transfer. After initial horizontal gene transfer from plants to bacteria, the acquired genes may be further spread to other bacteria.

Current scientific evidence indicates that the transfer of genes derived from GM plants into bacteria and their stable integration, either does not occur or, if it has occurred, it has been below the limit of detection in all the studies performed (see Keese, 2008; EFSA, 2009a and references therein; Brigulla and Wackernagel, 2010; Ma et al., 2011). The main barriers for horizontal gene transfer from plants to bacteria are the lack of efficient mechanisms of integration of unrelated chromosomal DNA and the limited potential for positive directional selection of the acquired recombinant gene-encoded traits.

The exposure of bacteria to the recombinant DNA fraction of maize GA21, the barriers limiting horizontal gene transfer, and the impact of hypothetical horizontal gene transfer in receiving environments are described below.

The probability and frequency of horizontal gene transfer of plant DNA (including the recombinant DNA fraction) to exposed bacteria is determined by (1) the concentration and quality of plant DNA accessible to bacteria in receiving environments, (2) the presence of bacteria with a capacity to develop competence for natural transformation, i.e., to take up extracellular DNA, (3) the ability for genetic recombination by which the plant DNA can be incorporated and thus stabilised in the bacterial genome (including chromosomes or plasmids), (4) the expression and the function of the protein in the bacterial recipient, and by (5) the selective advantage provided by the acquired recombinant gene-encoded traits.

The release and low-level temporal persistence of gene-sized plant DNA fragments is expected in environments where crops are grown and in gastrointestinal systems after consumption (EFSA, 2009a; Rizzi et al., 2012). The scope of this application is for food and feed uses, import, processing and cultivation of maize GA21. Therefore, the main exposure to DNA would occur in agricultural soils and gastrointestinal systems, mainly of animals fed maize GA21.

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[38] Technical dossier / Section D6

It is expected that bacteria in the digestive tract of humans, domesticated animals, and other animals feeding on maize GA21 will be exposed to low levels of fragmented products of the ingested DNA, including the recombinant genes (section 5.1.3). DNA is a component of many food and feed products derived from maize, but becomes substantially degraded during food/feed processing, and in the process of digestion in the human or animal gastrointestinal tracts (Jonas et al., 2001; van den Eede et al., 2004; Ramessar et al., 2007). The DNA is increasingly degraded in the digestive tract, so no full-length genes from plants have been detected in the large intestine or in faeces (EFSA, 2009a and references therein). In *in vivo* experiments with broilers fed Bt-maize, the *cry1Ab* gene was degraded to fragments smaller than 500 bp along the digestive tract (Rossi et al., 2005). Similarly, Chambers et al. (2002) fed chickens with GM maize to explore the *in vivo* fate of the bacterial ampicillin resistance gene bla_{TEM} in bacteria and GM maize. The gene was found in the stomach contents, but not in the lower intestine of animals fed GM maize. In case of Roundup Ready maize (event 39T67), the presence of *epsps* genes due to feeding on the GM plant material was reported in soil micro-arthropods, nematodes, macro-arthropods and earthworms within a field where the crop was grown (Gulden et al., 2008; Hart et al., 2009b).

Soil bacteria may also be exposed to extracellular DNA released from plant cells into the soil environment throughout and after the growing season (reviewed by Levy-Booth et al., 2007). During active plant growth, free plant DNA may originate from sloughed off root cap cells (Hawes et al., 1990; de Vries et al., 2003) or necrotic root tissue infected by pathogens (Polverari et al., 2000; Kay et al., 2002). Pollen release at anthesis (de Vries et al., 2003; Webster et al., 2008) and DNA release from decomposing plant residue remaining in agricultural areas after harvest, and which is incorporated into the soil during tillage operations (Widmer et al., 1997; Ceccherini et al., 2003; Stotzky, 2004), can also contribute to the presence of plant DNA in soil later during the growing season. However, the vast majority of plant DNA is expected to be degraded shortly after harvest by plant and microbial DNases in the soil environment. Therefore, plant DNA is only a transient component of the total DNA pool in soil (Levy-Booth et al., 2007; Nielsen et al., 2007; Gulden et al., 2008). Gulden et al. (2008) did not observe accumulation of the *epsps* gene in the soil environment upon repeated cultivation of Roundup Ready maize (event 39T67). While adsorption to soil particles, particularly clay, can slow down DNA degradation, the vast majority is degraded shortly after harvest. It can therefore be concluded that the concentration of extracellular DNA fragments (including the m*epsps* gene of maize GA21) in gastrointestinal tracts, soil or other environments is relatively low.

Several bacterial species with the potential to develop competence for natural transformation (take up and recombine with extracellular DNA) belong to the common gut microbial community (Rizzi et al., 2008, 2012; EFSA, 2009a). However, competence development and transformation of such bacteria with genomic DNA of plants has not been observed in the lower gastrointestinal tract even with optimised model systems providing a selective advantage (Nordgård et al., 2007; EFSA, 2009a; Rizzi et al., 2012). In contrast, some studies have shown that introduced bacteria can be naturally transformed in the oral cavity of humans and animals (Duggan et al., 2000, 2003; Mercer et al., 1999a,b, 2001; Rizzi et al., 2012). Once the recombinant DNA is taken up, it must integrate into the recipient genome to persist during host replication. The likelihood of gene integration is influenced by the gene context (i.e., the surrounding/neighbouring sequences) of the recombinant gene(s) in the plant (EFSA, 2009a).

Homologous recombination efficiently facilitates integration of non-mobile, chromosomal DNA fragments into bacterial genomes (see EFSA, 2009a and references therein). This process depends on the presence of stretches of identical DNA sequences between the recombining DNA molecules. In addition to substitutive recombination events, where only the homologous genes are replaced, homologous recombination can also facilitate the insertion of non-homologous DNA sequences into bacterial genomes (additive recombination) if the flanking regions share sufficient sequence similarity.

The *nos* terminator sequence in maize GA21, which has been derived from *A. tumefaciens* and which occurs in multiple copies in maize GA21, theoretically could provide sufficient DNA similarity for homologous recombination to take place in strains of *Agrobacterium* carrying the *nos* gene.

In addition to homology-based recombination processes, non-homologous recombination events, that do not require the presence of DNA similarity between the recombining DNA molecules, are also theoretically possible. Non-homologous recombination has rarely been described in bacteria. In one study, the transformation rates for non-homologous recombination-based gene acquisitions were 10^{10}-fold lower than for homologous recombination-based gene acquisitions (de Vries et al., 2004; Hülter and Wackernagel, 2008; EFSA 2009b). Non-homologous recombination events have not been detected in studies that have exposed bacteria to high concentrations of DNA from GM plants (see EFSA 2009b). Non-homologous recombination scenarios for the m*epsps* gene in maize GA21 are therefore not further considered here.

Expression of the acquired DNA is considered a prerequisite to produce a risk-relevant change in the phenotype of the transformed bacteria. The m*epsps* gene in maize GA21 is regulated by an eukaryotic plant promoter and it is unlikely that they are efficiently expressed.

Bacterial communities are continually exposed to a high diversity of DNA sources in the environment. Therefore, a positive directional selection is considered to be required for rare horizontal gene transfer events to become biologically meaningful in the risk assessment.

The horizontal gene transfer event hypothesised above is not likely to be maintained in bacterial populations due to the lack of selective advantage for gene transfer recipients in case they would be able to express their acquired recombinant genes. The hypothesised low level exposure of environmental bacterial communities to the maize GA21 m*epsps* gene must be seen in the context of the natural occurrence and level of exposure to other sources of similar genes to which bacterial communities are continually exposed. In the unlikely event that the above mentioned genes and regulatory elements are taken up by bacteria, no selective advantage is anticipated, because *epsps* genes are already occurring in various bacterial species in the environment, except in the presence of glyphosate.

The unlikelihood of double homologous recombination, the wide environmental presence of genetically diverse natural variants of the recombinant DNA coding sequences, and the absence of an identified plausible selective advantage, except in the presence of glyphosate, that would be provided to hypothesised transformed bacteria, suggest it is highly unlikely that the recombinant DNA will transfer and establish in the genome of bacteria in the human and animal digestive tract or in the environment (EFSA, 2009a). Hence, in the rare but theoretically possible case of transfer of the m*epsps* gene from maize GA21 to bacteria, no novel property would be introduced into the soil bacterial community and thus no positive selective advantage, except in the presence of glyphosate, that would not have been conferred by natural gene transfer between bacteria would be provided.

In its evaluation, the EFSA GMO Panel did not identify properties with the inserted DNA in maize GA21 that would change its likelihood of horizontal transfer compared to other plant genes. A selective advantage of hypothesised rare horizontal transfer of the recombinant gene (m*epsps*) to environmental bacteria has not been identified. Therefore, the EFSA GMO Panel concludes that the recombinant DNA in maize GA21 does not represent an environmental risk in relation to its potential for horizontal transfer to bacteria.

The conclusion of the EFSA GMO Panel is consistent with that of the CZ CA. The CZ CA concluded that "*the likelihood of horizontal gene transfer and subsequent expression of the mepsps gene in bacteria is extremely low*", and that "*the potential ecological impact due to gene transfer from GA21 to the soil microflora is negligible*". The CZ CA also stated that *the possibility of transfer to animals is extremely low. In the highly unlikely event that intact mepsps was transferred and expressed in animals the impact would be negligible because animals do not have the necessary substrates for the EPSPS enzyme*" (section 7.3 of the environmental risk assessment report of the CZ CA).

6.2.2.2. Plant to plant gene transfer and its consequences[39]

Maize is a cross-pollinating plant, relying on wind for the dispersal of its pollen. While maize pollen can be collected by honeybees and other insects, these pollinating insects play a minor role in the cross-pollination of maize plants (Eastham and Sweet, 2002; Malone and Burgess, 2009).

Compared to other wind-pollinated species, pollen grains of maize are relatively large (an average diameter of 90 μm) and heavy (0.25 μg) (Raynor et al., 1972; Di-Giovanni et al., 1995). Due to their characteristics, maize pollen grains settle to the ground rapidly (Aylor et al., 2003) and have usually a short flight range (Jarosz et al., 2005). Approximately 95-99 % of the released pollen is deposited within about 50 m from the source. However, vertical wind movements or gusts during pollen shedding can lift pollen up high in the atmosphere and distribute it over significant distances up to kilometres (Jarosz et al., 2005; Astini et al., 2009; Vogler et al., 2009; Hofmann et al., 2010). Concentrations of viable pollen considerably decrease with height (Aylor et al., 2006) and distance (Jarosz et al., 2005) from the source. Very low levels of cross-pollination can occur over distances up to kilometres under suitable climatic conditions (Bannert and Stamp, 2007; Delage et al., 2007; Langhof et al., 2010; Kawashima et al., 2011), but most cross-pollination events occur within 40 m of the pollen source (reviewed by Eastham and Sweet, 2002; Devos et al., 2005, 2009b; van de Wiel and Lotz, 2006; Hüsken et al., 2007; Langhof and Rühl, 2008; Sanvido et al., 2008; Ricroch et al., 2009; van de Wiel et al., 2009; Czarnak-Klos and Rodríguez-Cerezo, 2010; Riesgo et al., 2010).

Maize pollen is susceptible to desiccation, and water loss in pollen grains during dispersal reduce their ability to germinate on the stigma (Aylor, 2004). In addition, the water content of maize pollen affects its flight dynamics (Aylor, 2002, 2003; Aylor et al., 2003). During drying, the shape of maize pollen changes from a prolate spheroid to a crinkled, prismatic solid, and its density increases by approximately 16 %, and its settling speed decreases by approximately 34 %. These physical changes impact potential transport distances of pollen. In general, the lightest pollen will travel the longest distances, but it will be the least viable (Aylor, 2002).

The EFSA GMO Panel does not consider pollen dispersal and consequent cross-pollination as environmental hazards in themselves, and is primarily concerned with assessing the environmental consequences of transgene flow on ecosystems by considering the spread and fitness of hybrid and backcross progeny, as well as exposure to non-target organisms (section 6.2.4).

Theoretically, seeds originating from the cross-pollination of certain sexually compatible wild relatives can mediate the potential spread and establishment of hybrid and backcross progeny (Wilkinson et al., 2003; Morales and Traveset, 2008; Devos et al., 2009a). However, in the EU, there are no sexually cross-compatible wild relatives with which maize can hybridise and form backcross progeny (Eastham and Sweet, 2002; OECD, 2003). The only recipient plants that can be cross-fertilised by maize are other cultivated maize varieties and types (Devos et al., 2005, 2009b; van de Wiel and Lotz, 2006; Hüsken et al., 2007; Sanvido et al., 2008; Bitocchi et al., 2009; Ricroch et al., 2009; Czarnak-Klos and Rodríguez-Cerezo, 2010). Since the molecular analysis and food/feed safety evaluation did not raise safety concerns (sections 3 to 5; EFSA, 2007), the EFSA GMO Panel does not consider cross-pollination in maize an environmental risk, but an agricultural management and coexistence issue that is not within its remit.

Seed-mediated establishment of maize and its survival outside cultivation is rare in spite of extensive cultivation in many countries and accidental seed dispersal. Maize plants have lost their ability to release seeds from the cob, so most seed dispersal is due to harvesting and post-harvest activities of farmers. The occurrence of some GM maize plants outside cropped area has been reported in Korea and is attributed to seed spillage during import, transportation, storage, handling and processing (Kim et al., 2006; Lee et al., 2009; Park et al., 2010). However, survival of maize outside cultivation in Europe is limited by a combination of low competitiveness, absence of a dormancy phase, and susceptibility to plant pathogens, herbivores and cold climatic conditions. Furthermore, since these

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[39] Technical dossier / Sections D6 and D9.3

general characteristics are unchanged in maize GA21, it is considered very unlikely that it or its progeny will differ from conventional maize varieties in their ability to establish feral populations under European environmental conditions. The herbicide tolerance trait is not likely to provide selective advantages outside cultivation or other areas where glyphosate-based herbicides could be applied in Europe. Therefore, as for any other maize varieties (Raybould et al., 2011), maize GA21 plants are not likely to establish feral populations under European environmental conditions. The contribution of occasional feral GM maize plants to pollen flow into agricultural fields will be extremely small, compared to that from the crop. Moreover, 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 adjacent plants only at low levels (Palaudelmàs et al., 2009; section 6.2.1). In comparison with GM maize volunteers, the vigour of occasional feral GM maize plants will be reduced due to the less suitable habitat than agricultural fields.

In conclusion, since maize GA21 has no altered agronomic and phenotypic characteristics, except for the herbicide tolerance (section 6.2.1), the EFSA GMO Panel is of the opinion that the likelihood of unintended environmental effects as a consequence of spread of genes from maize GA21 is considered to be extremely low.

The conclusion of the EFSA GMO Panel is consistent with that of the CZ CA on maize GA21. The CZ CA concluded that "*maize has no sexually compatible wild or weedy species, therefore outcrossing of GA21 maize can only occur with other cultivated maize. If this occurred, the potential environmental impact of this maize would be comparable to that of GA21 maize*" (section 7.3 of the environmental risk assessment report of the CZ CA).

6.2.3. Interactions of the GM plant with target organisms[40]

Potential effects on target organisms due to the expression of the mEPSPS protein were not considered an issue by the EFSA GMO Panel, nor by the CZ CA and Member States, because the protein does not interact with any specific target organisms. The mEPSPS protein renders maize GA21 tolerant to the herbicidal active substance glyphosate, allowing direct application of glyphosate-based herbicides during cultivation. Glyphosate has a broad spectrum of target plant species, and potential impacts of the specific cultivation, management and harvesting techniques are considered in section 6.2.7.

6.2.4. Interactions of the GM plant with non-target organisms[41]

The potential of maize GA21 to have direct or indirect adverse effects on non-target organisms and the ecological functions they provide, such as pollination, biological control or decomposition (Sanvido et al., 2009; Arpaia, 2010), was evaluated by the EFSA GMO Panel. This evaluation covers the assessment of potential adverse environmental effects on non-target organisms due to intended and unintended changes in the GM plant (e.g., Hjältén et al., 2007; Desneux et al., 2010; Garcia-Alonso, 2010; Raybould et al., 2010; Arpaia et al., 2011). Intended changes in the GM plant are those that fulfil the original objectives of the genetic modification, whereas unintended changes are defined as consistent differences between the GM plant and its appropriate comparator, which go beyond the primary intended changes of introducing the transgene(s) (EFSA, 2010d,e). These changes may have consequences for the environment, and it is the potential adverse nature of these consequences that requires assessment. The EFSA GMO Panel follows two distinct yet complementary approaches for the risk assessment of potential adverse effects on non-target organisms (EFSA, 2010d,e).

6.2.4.1. Adverse effects on non-target organisms due to unintended changes in maize GA21

The molecular characterisation of the DNA insert and flanking regions of maize GA21 did not indicate unintended changes due to the insertion (section 3). Moreover, no biologically relevant differences in the composition of key analytes or agronomic and phenotypic characteristics were identified between maize GA21 and its near-isogenic line (EFSA, 2007). Therefore, the EFSA GMO Panel concludes that

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[40] Technical dossier / Section D9.4

[41] Technical dossier / Section D9.5

there are no indications of unintended changes in maize GA21 at the molecular, compositional and agronomic/phenotypic level.

In order to reliably conclude on potential adverse effects on non-target organisms due to unintended changes in maize GA21, the EFSA GMO Panel requested the applicant to review all *event*-specific studies on main functional groups of non-target organisms. The EFSA GMO Panel considers that *in planta* [event-specific] data on main functional groups of non-target organisms provide an additional indication on the possible occurrence of unintended changes in the GM plant (EFSA, 2010d,e).

Following the request of the EFSA GMO Panel, the applicant supplied supplementary information comprising both field- and laboratory-generated data. A field study was carried out in Spain in 2008 to determine the abundance of arthropods representative of the main functional groups present in maize[42]. Maize GA21 and its near-isogenic line were planted in a randomised complete block design with four replicates per treatment. Three different treatments were used, comprising maize GA21 treated with conventional herbicides, maize GA21 treated with glyphosate-based herbicides and the near-isogenic line of maize GA21 treated with conventional herbicides. No insecticide treatments were included. Data on abundance of non-target arthropods of the main functional groups present in maize fields were obtained. No significant differences were found in abundance or in phenology curves of herbivores (comprising arthropods of the orders: Orthoptera, Thysanoptera, Homoptera, Heteroptera and Coleoptera) and of plant-dwelling predators (comprising arthropods of the orders: Aranea, Deramptera, Thysanoptera, Heteroptera, Neuroptera and Coleoptera) between the different treatments. Collected data on parasitic hymenopteran wasps did not reveal significant differences. A similar field study, performed with maize Bt11 x MIR604 x GA21 and its near-isogenic line in Spain in 2008, showed no adverse effects on herbivorous and plant-dwelling predatory arthropods[43].

Because pollinators were not present in sufficient quantity in the 2008 field study with maize GA21 to enable statistical analysis, the applicant conducted a laboratory bioassay with honeybees (*Apis mellifera*)[44]. In this bioassay adult honeybees were fed pollen of maize GA21 and conventional maize with five replicate cages, each containing ten bees. Three dietary treatments were compared over a 96 hours observation period: bees were fed either maize GA21 pollen, non-GM maize pollen or a control consisting of untreated sugar solution. No statistically significant effects on the survival and feeding behaviour of bees were observed between the treatments.

The EFSA GMO Panel acknowledged the results of the acute toxicity study on honeybees, but considered that possible unintended sublethal environmental effects should also have been assessed. Therefore, a study on the effect of maize GA21 pollen on larval development of honeybees (including a prospective statistical power analysis) was requested from the applicant, together with an assessment of any other available information on sublethal effects.

To assess potential dietary effects of maize GA21 pollen and possible changes in plant-pollinator interactions, the applicant performed a laboratory/screenhouse study, in which the development and survival of honeybee larvae were studied, after exposure to maize GA21 pollen[45]. The maize GA21 pollen was prepared at a concentration of 2 mg of pollen per larval cell in combination with 30 % sucrose. Two additional treatments were: a negative control consisting of pollen from a near-isogenic line with a similar genetic background to maize GA21 in combination with 30 % sucrose, and a positive control (reference substance) consisting of pollen from a near-isogenic line with a similar genetic background to maize GA21 and 20 µg potassium arsenate, both applied at 2 mg of pollen per larval cell. The treatments were applied to four replicate groups of 20 honeybee larvae per group. Honeybee larvae (2-3 days old) were fed with a single dose of the appropriate diet at initiation and both development and survival were measured during larval and pupal development. Development appeared not to differ between the maize GA21 pollen treatment and the negative control treatment,

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[42] Additional information received on 17 February 2010 / Question 4 / Pages 10-15 / Appendix 5

[43] Additional information received on 21 July 2010 / Question 1 / Page 5 / Appendix 1: de la Poza (2010)

[44] Additional information received on 17 February 2010 / Question 4 / Pages 10-15 / Appendix 6

[45] Additional information received on 4 October 2011 / Question 3 / Page 5 / Appendix 2: Richards (2011)

but no statistical analysis was reported to substantiate this. Larvae from both treatments started emergence on the same day. The last larva from the negative control treatment emerged two days after the last larva emerged from the maize GA21 treatment. A statistically significant difference in the survival of larvae was observed between treatments, with a slightly higher survival (92.7 %) in the larvae exposed to maize GA21 pollen than in the larvae exposed to pollen of the negative control (81.4 %).

The EFSA GMO Panel noted that the applicant did not provide as requested the effect size that the experiment was designed to detect, did not to conduct the prospective power analysis the EFSA GMO Panel requested prior to the experiment and did not comment on the adequacy of the experiment to detect such an effect. Under the updated EFSA GMO Panel guidance for the environmental risk assessment of GM plants (EFSA, 2010e), future applications will require a mandatory prospective power analysis for all such studies, although in this case the EFSA GMO Panel noted that the study used a standard protocol with slightly increased replication. Despite these inadequacies, the EFSA GMO Panel notes that both treatments had survival greater than the control data quality objective of 80 % and that the levels of survival observed here are consistent with those reported in similar studies considered in previous GM plant applications for cultivation (EFSA, 2009c, 2011e). Therefore, the EFSA GMO Panel agrees with the conclusion of the applicant that no adverse effects on development or survival were observed in honeybee larvae treated with pollen from maize GA21 relative to the negative control treatment under the conditions of the study.

The studies on non-target organisms, supplied by the applicant, showed no adverse effects of maize GA21 or its pollen on different types of non-target herbivores, plant-dwelling predators or pollinators. Based on the evidence provided by the applicant and relevant scientific literature on maize GA21, the EFSA GMO Panel concludes that there are no indications of adverse effects on non-target organisms due to unintended changes in maize GA21. Since there are no indications of unintended changes, the EFSA GMO Panel considers *trait*-specific information appropriate to assess whether maize GA21 poses a risk to non-target organisms. The assessment of potential adverse effects on non-target organisms due to the expression of the mEPSPS proteins is described in the below section 6.2.4.2.

6.2.4.2. Adverse effects on non-target organisms due to the expression of the mEPSPS protein

Based on the mode of action of the mEPSPS protein and the history of safe use of maize GA21 and other glyphosate tolerant crops, it is unlikely that the expression of this protein in glyphosate tolerant crops will cause direct adverse effects on non-target organisms (see e.g., EFSA, 2009c for maize NK603; CERA, 2010). The mEPSPS protein shares no significant homology with known toxic proteins (section 4; EFSA, 2007) and is more than 99.3 % homologous to maize EPSPS. EPSPS proteins are ubiquitous in plants and microorganisms (CaJacob et al., 2004; CERA, 2010).

The applicant concluded that the probability of direct adverse effects of maize GA21 on non-target organisms due to the expression of the mEPSPS protein is very low, as no biologically relevant differences in the composition of key analytes or agronomic characteristics were identified between maize GA21 and its conventional counterpart, and because the molecular characterisation of the DNA insert and flanking regions of maize GA21 did not raise safety concerns (section 4; EFSA, 2007).

The lack of toxicity of the mEPSPS proteins to honeybees[46], rats[47] and broiler chickens[48] fed diets containing maize GA21 material was shown in toxicity studies and nutritional equivalence studies (section 4; EFSA, 2007 for further details).

Higher-tier studies conducted with EPSPS-expressing crops such as maize GA21[49], maize NK603 (Rosca, 2004; Reyes, 2005; Rodriguez et al., 2006; Schier, 2006; Albajes et al., 2008, 2009, 2010;

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[46] Additional information received on 17 February 2010 / Question 4 / Pages 10-15 / Appendix 6

[47] Technical dossier / Section D7.8.4 / Appendix 25

[48] Technical dossier / Section D7.9 / Appendix 26

[49] Additional information received on 17 February 2010 / Question 4 / Pages 10-15 / Appendix 5 // Additional information received on 21 July 2010 / Question 2 / Page 6 / Appendix 4

EFSA, 2009c), maize MON 88017 (EFSA, 2011e) and soybean GTS 40-3-2 (Buckelew et al. 2000; Bitzer et al., 2002; Jasinski et al., 2003) confirmed that the exposure of non-target organisms to the EPSPS proteins found in these crops poses no potential hazard, supporting the conclusions of lower-tier studies. The higher-tier study of Jasinski et al. (2003) suggested that the abundance of some beneficial organisms decreased in fields with glyphosate tolerant crops compared to fields planted with conventional crops. These reductions do not seem to be directly associated with the expression of EPSPS protein in herbicide tolerant crops, but are likely to be a consequence of changes in weed populations caused by different weed management regimes.

Available evidence showed no adverse effects on different types of non-target organisms due to the expression of EPSPS proteins in glyphosate tolerant crops. The EFSA GMO Panel notes that maize GA21 and other glyphosate tolerant crops have been extensively cultivated in the USA and elsewhere for several years, and is not aware of any reports of direct effects on non-target organisms due to the expression of EPSPS proteins. Recent publications confirmed that there is no evidence that glyphosate tolerant crops have a direct effect on biological diversity or species abundance within cropped fields due to the expression of EPSPS proteins (Firbank et al., 2003a; Cerdeira and Duke, 2006, 2007, 2010; Albajes et al., 2008, 2009, 2010; Owen, 2008; CERA, 2010).

Since the mEPSPS protein has no insecticidal activity against non-target organisms, the EFSA GMO Panel does not require lower-tier studies for the evaluation of potential adverse effects on non-target organisms due to the expression of the mEPSPS protein; testing of non-target organisms without a sound hypothesis would add little to the overall risk assessment.

The conclusion of the EFSA GMO Panel on the absence of adverse effects of maize GA21 to non-target organisms due to the expression of the mEPSPS protein is consistent with the evaluation carried out by the CZ CA on maize GA21. The CZ CA agreed with the applicant's statement that "*GA21 maize has no effects on NTOs* [non-target organisms]", but pinpointed that "*the change in herbicide management associated with GA21 maize cultivation should be also considered*" (section 7.5 of the environmental risk assessment report of the CZ CA).

Effects of the cultivation of maize GA21 and the use of glyphosate are considered in section 6.2.7.

6.2.5. Effects on human and animal health[50]

The molecular analysis and the food and feed safety assessment of maize GA21 did not raise safety concerns for human and animal health (sections 3 to 5; EFSA, 2007). In its previous Scientific Opinion on maize GA21 (EFSA, 2007), the EFSA GMO Panel concluded that "*maize GA21 is as safe as its non genetically modified counterparts with respect to potential effects on human and animal health conventional maize*", and that "*maize GA21 is unlikely to have any adverse effect on human and animal health in the context of its intended uses*".

6.2.6. Interactions with biogeochemical processes and the abiotic environment[51]

The mEPSPS protein expressed in maize GA21 can be introduced into the soil via physical damage to plant tissues, via decomposition of shed root cells during plant growth, via decomposing plant residues remaining in fields after harvest, which might be incorporated into the soil during tillage operations (Stotzky, 2004), and possibly via root exudates (e.g., Saxena et al., 2002, 2004; Icoz and Stotzky, 2007; Icoz et al., 2008), resulting in exposure of non-target soil organisms to the mEPSPS protein. Indirect exposure through manure and faeces from animals fed maize GA21 was also considered, though most of the mEPSPS protein would be degraded by enzymatic activity in the intestinal tract and subsequently by microbial processes in the manure.

No direct effects on biogeochemical processes and the abiotic environment of maize GA21 due to the expression of the mEPSPS protein have been reported by the applicant. The CP4 EPSPS and mEPSPS

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[50] Technical dossier / Sections D9.6 and D9.7

[51] Technical dossier / Sections D9.8 and D9.10

proteins were shown not to alter key soil microbial processes, such as carbon and nitrogen transformation, via lower- and higher-tier studies performed with maize NK603 in France (Philippot et al., 2006), glyphosate tolerant maize in Canada (Hart et al., 2009), or with glyphosate tolerant maize and soybean in the USA (Liphadzi et al., 2005). Because the mEPSPS protein of maize GA21 is similar to the EPSPS proteins found in plants and microorganisms (CERA, 2010), it is unlikely that it will affect the microbial community and hence biogeochemical processes adversely. Likewise, the expression of the newly introduced trait, of which the wild-type variants are naturally occurring in the soil environment, is not expected to alter the natural interactions of maize plants with the abiotic environment. The EFSA GMO Panel is not aware of any reports of effects on biogeochemical processes and the abiotic environment due to this trait (Dunfield and Germida, 2004; Cerdeira and Duke, 2006; Powell et al., 2007; CERA, 2010).

The conclusion of the EFSA GMO Panel on the absence of potential adverse effects of maize GA21 on biogeochemical processes and the abiotic environment due to the expression of the mEPSPS protein is consistent with the evaluation carried out by the CZ CA on maize GA21. The CZ CA concluded that "*there is negligible likelihood of immediate or delayed adverse impacts on biogeochemical processes resulting from cultivation of Event GA21 maize*" (section 7.7 of the environmental risk assessment report of the CZ CA).

6.2.7. Impacts of the specific cultivation, management and harvesting techniques[52]

The EFSA GMO Panel considers that the use of glyphosate, a broad-spectrum, non-selective herbicide associated with the cultivation of genetically modified herbicide tolerant (GMHT) maize GA21 in cropping systems is a change in the cultivation and management of this maize compared to conventional maize. Currently, the control of weeds in maize is mostly achieved by using pre-emergence soil acting residual herbicides and/or post-emergence selective herbicides in Europe. Very little control using cultural or mechanical means alone, without the use of selective herbicides, is applied in current European maize cropping systems (Bastiaans et al., 2008), though some has been suggested particularly for continuous maize or organic systems (Dewar, 2009; Meissle et al., 2010).

6.2.7.1. Interplay between the legislation for GMOs and plant protection products

Directives 2001/18/EC and 91/414/EEC (which was repealed by Regulation (EC) No 1107/2009 on 14 June 2011) are both relevant for the risk assessment of GMHT crops and their associated weed control management practices (EC, 2008b; EFSA, 2008a; Ehlers, 2011). The registration and use of herbicidal active substances in formulations in the EU was covered by Directive 91/414/EEC (which is now replaced by Regulation (EC) No 1107/2009) as operated by individual Member States. Where GMHT plants rely on specific herbicides as an integral part of a weed management strategy, an environmental risk assessment must also consider their potential impact on biodiversity under Directive 2001/18/EC. In the current legislation governing the registration of plant protection products in Europe, the environmental risk assessment of pesticides includes an assessment of impacts on certain non-target organisms (such as fish, Daphnia, algae, birds, mammals, earthworms, bees and beneficial arthropods and non-target plants) and studies of residual activities in soil and water (cf., environmental fate) (Streloke, 2011). On the basis of environmental impact indices, a large number of authors have claimed that some of the herbicidal active substances used on GMHT crops (e.g., glyphosate) have reduced environmental impacts compared with those applied on their conventional counterparts (Nelson and Bullock, 2003; Peterson and Hulting, 2004; Brimner et al., 2005; Brookes and Barfoot, 2006; Leroux et al., 2006; Kleter et al., 2007; Bonny, 2008, 2011; Devos et al., 2008; Arregui et al., 2010; Mamy et al., 2010). However, the environmental impact indices used for these calculations are generally based on residual, persistence and ecotoxicity characteristics, and do not relate to the efficacy and hence the biodiversity impact of herbicides (e.g., van der Werf, 1996; Reus et al., 2002). Indeed, the environmental risk assessment under Directive 91/414/EEC did not include studies of impacts on biodiversity within crops and changes in agro-ecosystems, which are required

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[52] Technical dossier / Section D9.9 / Appendix 27 // Additional information received on 13 July 2009 / Appendices 4, 7, 8 and 10 // Additional information received on 17 February 2010 / Question 6 / Pages 22-27 / Appendix 7 // Additional information received on 21 July 2010 / Question 1 / Pages 5-8 / Appendices 3, 4, 5 and 7

under Directive 2001/18/EC in relation to GM crops. Due to these different legal requirements, a herbicide used on a GMHT crop is currently assessed differently from the same herbicide used on non-GMHT crops (e.g., imidazolinone tolerant crops) and conventional crops. The assessment of GMHT crops regimes includes evaluating potential effects on farmland biodiversity, while this is not a requirement for non-GM crop herbicide regimes (ACRE, 2007a; Morris, 2007; Sanvido et al., 2007, 2011a,b; Ehlers, 2011). While an assessment of indirect effects of herbicidal active substances on biodiversity was not required for the risk assessment of pesticides under Directive 91/414/EEC, the new Regulation (EC) No 1107/2009, concerning the placing of plant protection products on the market, explicitly mentions biodiversity as a protection goal (Streloke, 2011). Moreover, Directive 2009/128/EC aims to strike a new balance between food security and the support of biodiversity by promoting the sustainable use of pesticides.

6.2.7.2. Herbicide regimes in maize cropping systems

Herbicide regimes in conventional maize in Europe

The sensitivity of maize to early weed competition is well-understood and the need for efficient weed control in the early maize growth stages often requires herbicide use with soil (residual) activity. Maize is very delicate in its early growth stages; it is very susceptible to competition for resources such as water, nutrients and light (Johnson et al., 2000; Lehoczky et al., 2004; Dewar, 2009; Teasdale and Cavigelli, 2010). Therefore, it is important to protect the early growth stages from weed interference until crop canopy development naturally limits the emergence of weeds (this usually takes place around the 8th leaf stage of maize). Three different herbicidal weed management strategies are possible in conventional maize for the control of annual and perennial grass and broadleaf weeds (Champion et al., 2003; Lehoczky et al., 2004; Beckie et al., 2006):

(1) application(s) pre-emergence of the crop;

(2) application(s) early post-emergence, ideally in the 2nd to the 4th leaf stage of maize;

(3) sequential applications, where a combination of herbicides with soil (residual) activity is applied pre-emergence followed by a mixture of post-emergence herbicides with foliar activity.

The choice of herbicides, applied alone or in tank mixtures, is driven by the need to cope with a wide spectrum of weeds present, which can vary greatly according to climate, soil type, season, field history, rotation, weed life cycles and cultivation practices (Dewar, 2010; Meissle et al., 2010).

Glyphosate is a broad-spectrum contact systemic herbicidal active substance used for the control of most annual and many perennial weeds (Duke and Powles, 2008b), but with little or no soil acting (residual) properties. It is absorbed by green leaves and stems from where it is translocated into plant tissues via the apoplast and the symplast. Glyphosate uptake by roots is minimal, so there is no long-term exposure of weeds to herbicidal activity. In the EU, glyphosate is currently used in conventional cropping by some farmers as a pre-sowing or pre-emergence herbicide. It is used pre-emergence of the crop for removing emerged weeds (Monsanto, 2010). In some situations, glyphosate is applied in an emerged crop as a band application between crop rows with the herbicide application being directed away from crop foliage to avoid crop injury (Duke and Powles, 2008b; Dewar, 2009), or it is used through weed wipers when weeds (especially perennials) are taller than the crop[53].

Glyphosate-based herbicide regimes in genetically modified herbicide tolerant (GMHT) maize

Glyphosate-based herbicides will be applied post-emergence with little or no injury to the GMHT crop. In contrast to selective herbicides that need to be applied when weeds are still in a young development stage, weed management strategies relying on glyphosate enable growers to delay the post-emergence application of a broad-spectrum herbicide until after weed emergence (Gianessi, 2005; Cerdeira and Duke, 2006). The efficacy of glyphosate at controlling weeds is less dependent on weed

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[53] http://www.monsanto-ag.co.uk/content.output/181/181/Roundup/Application%20Information/Weed%20wipers.mspx

size, so that glyphosate can be used up to a later growth stage for weeds, offering a greater flexibility in timing of weed management (Gianessi, 2008; Dewar, 2009, 2010). It is expected that the introduction of glyphosate in GMHT maize will replace or reduce the use of other herbicidal active substances used pre-emergence or early post-emergence of the crop (Dewar, 2010). However, the control of larger and perennial weeds will require higher application rates.

Several strategies have been proposed for controlling weeds in GMHT maize depending upon the spectrum and density of weeds present at or just after sowing (reviewed by Beckie et al., 2006; Devos et al., 2008; Dewar, 2009, 2010)[54].

(1) A single application (or sequential applications) of glyphosate-based herbicide alone, with no use of pre-emergence herbicides. However, note that field trials have shown that the use of glyphosate alone, applied post-emergence on one occasion at the recommended application rates can be inadequate to control all the weeds present throughout a full growing season (Gianessi et al., 2002; Gower et al., 2002, 2003; Grichar and Minton, 2006; Parker et al., 2006)[55]. In a trial of glyphosate tolerant maize (event NK603) in the Czech Republic, Soukup et al. (2008) only achieved acceptable herbicide efficacy for a single application of glyphosate when applied at dose rates above 1,440 g/ha active substance (ai). Additionally, the achievement of acceptable levels of herbicide efficacy depends upon the correct timing of glyphosate application (Johnson et al., 2000; Thomas et al., 2004, 2007; Beckie et al., 206). If the first treatment is applied too early, then weeds emerging after the application will remain unaffected. These weeds can not only reduce crop yield by competing for resources, but also increase weed pressure in subsequent years (Myers et al., 2005). Recommended strategies to avoid weed re-infestation involve the use of two post-emergence applications of glyphosate (Gower et al., 2002, 2003; Gehring and Mülleder, 2004). In case of maize NK603, both Soukup et al. (2008) and Verschwele and Mülleder (2008) achieved optimal herbicide efficacy by providing a double application of glyphosate, each with a dose rate of 1,080 g/ha ai. However, the increased frequency of glyphosate use could be a more important factor than glyphosate rate in favouring selection for glyphosate resistance, as shown by Preston et al. (2009) in *Lolium rigidum*.

(2) Application of a glyphosate-based herbicide in combination with other herbicides. A delay in the first glyphosate application can lead to yield reductions if there is an extended period of early weed competition (Gower et al., 2002, 2003; Champion et al., 2003; Cox et al., 2006). To limit such early-season competition and avoid maize yield losses, another strategy involves the use of other herbicides, especially residual herbicides applied pre-emergence (Grichar and Minton, 2006; Nurse et al., 2006). Glyphosate has little or no residual activity when applied to the soil surface where it strongly binds to soil particles. Moreover, glyphosate uptake by the plant roots is minimal. If glyphosate is applied pre-emergence, then there would be no long-term exposure of weeds to herbicidal activity, and weeds germinating subsequent to application would remain unaffected. Therefore, specific suggestions for this second strategy (Thomas et al., 2004, 2007; Parker et al., 2006) are for the use of pre-emergence residual conventional herbicides followed by a single delayed post-emergence application of glyphosate, which may eliminate the need for a second application. For this weed control strategy, Dewar (2010) recommended that the pre-emergence herbicide be applied at a reduced dose rate and that glyphosate be applied at a dose rate of 1,080 g/ha ai.

(3) A single application of a glyphosate-based herbicide in combination with other compatible post-emergence herbicides with residual activity. This strategy has been recommended by Gianessi (2008) and Soukup et al. (2008) specifically for receiving environments in which early post-emergence herbicides are predominantly used instead of pre-emergence herbicides. If applied sufficiently early, this strategy can eliminate early-season weed competition (Johnson et al., 2000; Thomas et al., 2007; Dill, 2005; Tharp et al., 2004; Grichar and Minton, 2006; Parker et al., 2006; Young, 2006; Zuver et al., 2006) and facilitate the control of weeds that are less susceptible to

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[54] Additional information received on 13 July 2009 / Question 3 / Pages 2-8 / Appendix 4

[55] Additional information received on 21 July 2010 / Question 2 / Pages 5-6 / Appendix 3

glyphosate (e.g., Norsworthy et al., 2001; Soukup et al., 2008). Based on field studies conducted at 35 sites throughout the north-central USA, Gower et al. (2003) concluded that the optimum timing for a single glyphosate application to avoid maize yield loss is when weeds are less than 10 cm in height, no later than 23 days after maize planting, and when maize growth was not more advanced than the 4th leaf stage. The use of glyphosate-based herbicides at a dose rate of 1,080 g/ha ai in conjunction with the soil-active herbicidal active substance acetochlor resulted in high herbicide efficacy in field trials in the Czech Republic (Soukup et al., 2008).

(4) Several applications of broad-spectrum herbicides, including glyphosate. In cases of high weed pressure, other chemical-containing weed control strategies suggested to include the sequential application of glyphosate in conjunction with residual herbicides applied pre-emergence or early post-emergence (Thomas et al., 2007; Dewar, 2010); or the use of other broad-spectrum herbicides (such as dicamba) in combination with glyphosate (Dewar, 2009, 2010; Green and Castle, 2010; Green, 2011; Green and Owen, 2011).

In conclusion, pre- or post-emergence residual herbicides could be used in combination with the post-emergence application of glyphosate around the 4th and 6th leaf stage, in order to give optimum control and yield protection during the most vulnerable maize growth stages (Soukup et al., 2008; Dewar, 2009).

Recommended herbicide regimes for maize GA21 by the applicant

In its application under Regulation (EC) No 1829/2003, the applicant provided provisional recommendations on the application rates of glyphosate on maize GA21[56]. The applicant recommended "*herbicide regimes where the maximum total application rate of glyphosate is 2880 g/ha ai per year, administered in up to 2 applications per crop*", and clarified that "*application dose rates, ranging from 1440-2880 g/ha ai, are expected to provide effective control of difficult to control perennial weeds, whilst lower dose rates (generally 540-1440 g/ha ai) might be sufficient to give effective control of annual weeds in maize*". These proposed application rates are currently being reviewed by the applicant in relation to the chemical market dossier according to Annex III of Directive 91/414/EEC (which was repealed by Regulation (EC) No 1107/2009 on 14 June 2011), but are indicative of the range of application rates, mixtures and systems that might be applied in the future. Hence, the applicant suggested that an appropriate recommended rate of glyphosate should be determined by the weed species, density and growth stages, and by mixture patterns (according to local good agricultural practices).

Proportion of maize in crop rotations

The applicant noted that maize GA21 will be used by farmers as any other maize, appearing in rotational-planning adapted to each specific geographic region and subject to changes in rotation for economic and customer/stakeholder demand reasons[57]. Maize-based cropping systems, with different shares of maize in crop rotations, are dominant in European arable systems (FCEC, 2009; Meissle et al., 2010; Vasileiadis et al., 2011). In the northern EU region, maize is mostly cultivated as non-irrigated continuous silage maize or rotated with grasses. In the eastern EU region (e.g., Hungary, Romania), maize is mostly cultivated as grain maize in rotation programs after wheat such as soybean (or other plant from the leguminosae family)-wheat-maize-sunflower or wheat-wheat-maize-sunflower or wheat-wheat-maize-oilseed rape), or as non-irrigated continuous grain maize. In southwest EU (e.g., Portugal, Spain), grain maize is commonly irrigated and either grown continuously or rotated with other crops that need also irrigation. The usual rotation is with winter wheat, cotton or sugar beet, but other crops such as sunflower, alfalfa or tomatoes can appear in the scheme. In the southern region (e.g., Po Valley, Italy), grain maize irrigated and rotated (mainly with winter wheat or soybean) is the main system identified, while other important systems include silage maize rotated and irrigated, as well as continuous and irrigated grain maize (reviewed by Vasileiadis et al., 2011).

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[56] Additional information received on 13 July 2009 / Question 3 / Pages 8-13 / Appendix 4

[57] Additional information received on 4 April 2011 / Request 2.4 / Pages 26-28

Conclusion

EU countries show considerable variation in herbicide use in maize depending on the crop type (grain, forage, sweet, etc.), the weed species (including crop volunteers) present, meteorological and agro-environmental conditions, farming systems (including weed resistance evolution management, rotation systems), economics, the growing season, and farmers' behaviour. Herbicide regimes are influenced by weed species and biology, since not all weeds are equally susceptible to glyphosate (e.g., Norsworthy et al., 2001; Soukup et al., 2008) and by factors influencing integrated pest and crop management. Hence, where weeds are required for soil erosion management, cover or protection, or as refuges for beneficial insects, then management will vary accordingly. Variations between locally-adopted herbicide regimes and cultivation management (including conservation tillage) for GMHT maize would be expected, in response to these factors. Therefore, it is anticipated that glyphosate-based herbicide regimes will represent a mixture of the strategies outlined above, and involve different numbers of applications (single vs. sequential), doses, timing of application, and the use of other herbicides (including soil acting residuals) in association with glyphosate. Nonetheless, to ensure the safe use of the GMHT technology, it is important that individual farmers adopt integrated weed management strategies based on use of multiple options (cultural, mechanical and chemical). Oversimplification of weed management due to the introduction of glyphosate tolerant crops has occurred in the USA (Bonny, 2008, 2011).

6.2.7.3. Environmental impact of herbicide regimes used in GMHT cropping systems

The EFSA GMO Panel considers that since farming systems are highly dynamic, the introduction of widespread broad-spectrum herbicide-based systems may lead to changes in management. For the reasons stated below, the EFSA GMO Panel does not agree with the applicant's assessment for environmental impact of the specific cultivation, management and harvesting techniques, which was limited to statements that the pattern of the use of glyphosate on maize GA21: "*is similar to that for post-emergence selective herbicides ... currently used in non-glyphosate tolerant maize*" and that "*cultivation of Event GA21 maize will not lead to change in management techniques in terms of use of herbicides*".

It has long been recognised that the widespread use of herbicides in agriculture has resulted in serious declines in both plant and animal diversity in many farming areas (Krebs et al, 1999; Chamberlain et al., 2000; Donald et al., 2001; Marshall et al., 2001, 2003; Stoate et al., 2001; Robinson et al., 2002; Fried et al., 2009; Geiger et al., 2010; Storkey et al., 2011). Concern has been expressed that GMHT crops, through the in-crop repeated use of very effective broad-spectrum herbicides, will further deplete biodiversity in farmland (Marshall et al., 2001). It is expected that the long-term persistence of arable weeds in the soil seedbank will decline in less-weedy fields, while invertebrates, small mammals and seed-eating birds might be threatened by reduced food resources and/or foraging and nesting habitats (Watkinson et al., 2000; Gibbons et al., 2006; Butler et al., 2007). Arable weeds play an important role in supporting biological diversity and have numerous interactions with other organisms that depend on them for food and shelter, and some of these interactions can have direct effects on the functioning of the agro-ecosystem (Clergue et al., 2005; Moonen and Bàrberi, 2008; Bàrberi et al., 2010; Petit et al., 2011). Herbivores, predators and parasitoids associated to arable weeds may in turn mediate essential processes through the functioning of arable food webs (Norris and Kogan, 2000; Hawes et al., 2003, 2009; Marshall et al., 2003; Gibbons et al., 2006; Taylor et al., 2006; Hilbeck et al., 2008). For example, granivorous and omnivorous carabid beetles interact closely with the arable weed seedbank (Lundgren, 2009; Bohan et al., 2011). The role of post-dispersal seed predators and consumers, including generalist vertebrates (birds, rodents) and invertebrates (Coleoptera, Hymenoptera, earthworms, molluscs, etc.) on the regulation of weed populations is being increasingly recognised (Tooley and Brust, 2002; Bàrberi et al., 2010). Westerman et al. (2005) showed that predation by opportunist invertebrates can substantially reduce the surface weed seed stock ('biological weed control' service). The regulation and control of arthropod pest populations resulting from the activity of natural enemies is also an important ecosystem service in arable systems (Losey and Vaughan, 2006; Macfadyen et al., 2009; Sanvido et al., 2009).

There is extensive literature on the range of effects of the use of glyphosate and its associated weed control management practices in glyphosate tolerant crops (reviewed by Cerdeira and Duke, 2006, 2007, 2010; Dewar, 2010). Beneficial effects (e.g., increase in collembolans, reduction of soil erosion, reduction in virus infection) due to the retention of weed cover on the soil surface during the early growth of the crop have been reported (Brookes et al., 2003; Dewar et al., 2003; May et al, 2005). In addition, the use of a broad-spectrum herbicide to control both monocotyledons and dicotyledons within the maize phase of a rotation may be compensated by a reduction in herbicide control of dicotyledonous weeds in other crops within the rotation, particularly if these are also cereals (Heard et al., 2005), although this effect cannot be generalised. Furthermore, the use of glyphosate allows greater adoption of no- or reduced-tillage systems (Locke et al., 2008; Givens et al., 2009b). These systems contribute to different extent to reductions in soil erosion, fossil fuel use, carbon dioxide emissions, nitrogen and pesticide leaching, and in loss of soil moisture, and to improved soil structure (Baylis, 2000; Cerdeira and Duke, 2006, 2007, 2010; Dewar, 2010; Basso et al., 2011; Carpenter, 2011). The abundance of soil-dwelling carabid beetles and spiders has been shown to increase in no- or reduced-tillage systems, as weeds provide a more favourable habitat for predators, such as carabids or spiders, or because more abundant prey, such as Collembola, are available (Witmer et al., 2003; Hough-Goldstein et al., 2004; Rodríguez et al., 2006; Schier, 2006). A life-cycle assessment in which the risks of conventional sugar beet agricultural practices were compared with those that might be expected if GMHT sugar beet was grown, suggested that growing GMHT sugar beet would be less environmentally harmful than its conventional counterpart (Bennett et al., 2004). Glyphosate has also been shown to be more environmentally and toxicologically benign than many of the herbicidal active substances that it replaces (reviewed by Cerdeira and Duke, 2006, 2007, 2010; Carpenter, 2011; see also[58]).

On the negative side, there is evidence that, depending upon the specific herbicide regimes applied at the farm level, the cultivation of GMHT crops may: (1) reduce farmland biodiversity, (2) induce changes in botanical diversity due to weed shifts, with the selection of weed communities mostly composed of tolerant species, (3) select for glyphosate resistant weeds, and (4) impact soil microbial communities (see also EFSA, 2009c, 2011e). These potential adverse indirect environmental effects of the cultivation of maize GA21 are discussed below.

Impact on farmland biodiversity

A few studies have assessed the impact of glyphosate-based herbicide regimes used in GMHT maize cultivation in Europe (Soukup et al., 2008; Verschwele and Mülleder, 2008; Albajes et al., 2008, 2009, 2010, 2011; Szekeres et al., 2008; Thieme, 2010). In addition, research projects such as the project on Botanical and Rotational Implications of Genetically modified Herbicide Tolerance in winter oilseed rape and sugar beet (BRIGHT) (Sweet et al., 2004; Lutman et al., 2008); the Farm Scale Evaluations (FSEs) (Firbank et al., 2003a,b) in the United Kingdom; and the study of the National Environmental Research Institute (NERI) in Denmark (e.g., Strandberg and Pedersen, 2002) have considered the impact of more general GMHT cropping systems and their associated herbicide regimes on farmland biodiversity. Additionally, there are some other studies of herbicide tolerant crops in European countries that have compared the environmental impact of conventional production systems with that of GMHT cropping systems (Madsen and Jensen, 1995; Bückmann et al., 2000; Coyette et al., 2002).

Indirect effects on farmland biodiversity associated with the use of glufosinate-ammonium- and glyphosate-based herbicides in GMHT cropping systems, including maize, were studied extensively in the FSEs (Firbank et al., 2003a). Results showed that herbicide regimes used with glufosinate-ammonium tolerant maize had less adverse impact on farmland biodiversity, compared to non-GM maize treated with conventional herbicides. In the maize growing season, the weed density in

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[58] Giesy et al., 2000; Wauchope et al., 2002; Nelson and Bullock, 2003; Solomon and Thompson, 2003; Peterson and Hulting, 2004; Brimner et al., 2005; Mamy et al., 2005; Screpanti et al., 2005; Vereecken, 2005; Brookes and Barfoot, 2006; Leroux et al., 2006; Kleter et al., 2007, 2008; Borggaard and Gimsing, 2008; Bonny, 2008, 2011; Devos et al., 2008; Duke and Powles, 2008b; Gardner and Nelson, 2008; Klier et al., 2008; Shipitalo et al., 2008; Struger et al., 2008; Arregui et al., 2010; Dewar, 2010; Mamy et al., 2010

glufosinate-ammonium tolerant maize was approximately two to three fold higher throughout the season, and biomass was 1.85-fold higher than in conventionally-managed maize. Due to the greater weed control exerted by conventional herbicide regimes, as compared with those used with glufosinate-ammonium tolerant maize, biomass of dicotyledonous weeds and counts of their seed-rain were greater in GMHT maize (Heard et al., 2003a,b). There were few effects on major groups of invertebrates, though there were more soil-dwelling detritivores in glufosinate-ammonium tolerant maize, especially in August, and more herbivores and their parasitoids in June (Hawes et al., 2003). In July, the seed feeding carabid *Harpulus rufipes* was more frequent in glufosinate-ammonium tolerant maize fields (Brooks et al. 2003). Consumer-resource ratios were similar between herbicide regimes, except that there were more invertebrate predators per herbivore in glufosinate-ammonium tolerant maize. In glufosinate-ammonium tolerant maize, weed seed rain, important in the diets of 17 granivorous bird species, was higher than in conventionally-managed maize, though the difference was only significant for the following seven species: *Pedrix pedrix*, *Columba oenas*, *Columba palumbus*, *Carduelis chloris*, *Pyrrhula pyrrhula*, *Emberiza schoeniclus* and *Emberiza cirlus* (Gibbons et al., 2006). In subsequent conventional crops, the beneficial effect of herbicide regimes was detectable in the weed soil seedbank. Soil seedbanks following glufosinate-ammonium tolerant maize were 1.23-fold higher than following conventional maize for both the first and second years (Firbank et al., 2005a). While long-term effects on farmland biodiversity have been predicted at the landscape level due to the continuous cultivation of GMHT crops in association with the exclusive use of glyphosate-based herbicides (Heard et al., 2005, 2006; Squire et al., 2009), such effects have not been confirmed by field data. In the second year of maize cultivation, there was no overall trend of herbicide regime ratios being greater or smaller when taken across taxa (Heard et al., 2006).

Caution is required when interpreting, extrapolating and scaling up the observations made under the conditions of the FSEs. First, the GMHT maize used in the FSEs was tolerant to the herbicidal active substance glufosinate-ammonium, whereas maize GA21 will be used in association with glyphosate. Glyphosate is a very effective broad-spectrum herbicidal active substance that provides more consistent control than glufosinate-ammonium in particular cases (see e.g., Leroux et al., 2006; Zuver et al., 2006). Glufosinate-ammonium behaves likes a contact herbicide, so unlike glyphosate, it must be applied to small weeds and is not as effective on perennials that require significant translocation for complete control (Green, 2011; Green and Owen, 2011). Second, herbicide regimes applied in non-GMHT maize included the herbicidal active substances atrazine, simazine and cyanazine in the FSEs (Champion et al., 2003). Considering that these herbicidal active substances have been withdrawn from the EU market, further analysis of the FSE data was deemed necessary. The reanalysis indicated that the replacement of triazine herbicides by less efficient conventional herbicides slightly reduced the net beneficial effect of herbicide regimes in GMHT maize, but did not eliminate it (Perry et al., 2004; Brooks et al., 2005). Third, the herbicide regimes used with glufosinate-ammonium tolerant maize in the FSEs might not fully reflect real agricultural practice, as the application of glufosinate-ammonium-based herbicides was limited to a single spray applied at dose rates lower than 0.800 kg/ha ai in most cases (Champion et al., 2003). In practice, however, it is reasonable to assume that other herbicide regimes than the one used in the FSEs will be implemented, resulting in a different impact on farmland biodiversity. Whilst the impact of glyphosate tolerant maize was not tested in the FSEs, it has been suggested that it might be similar to that which occurred in glyphosate tolerant sugar beet (Dewar, 2010). Reductions in the number of weeds in glyphosate-treated sugar beet, compared with conventionally-treated sugar beet, resulted in significant reductions in weed biomass, and in subsequent weed seed production later in the season and in the following crops (Dewar et al., 2005).

The above studies confirm that effects on arable weed populations, and hence farmland biodiversity, are highly dependent on the management of the herbicides in the GMHT and conventional crop production systems and on the herbicides used in both systems. The extent and direction of the effects of weed management on weeds and invertebrates is dependent on the relative efficacy of the existing conventional regimes and the forthcoming GMHT herbicide regimes. Extensive research has shown that impacts on biodiversity also depend greatly upon the management of crops, rotations, and upon the provision of forage and habitat resources across the entire farmed landscape (Firbank et al., 2003b). Here, crop management includes the dose applied, the time and the frequency of applications, both of the specific non-selective and of other herbicides (Champion et al., 2003). Timing of application is particularly important, since with broad-spectrum herbicides, application is often delayed until a later plant growth stage than is the case with the more selective herbicides associated with conventional crops. The higher mortality of larger (reproductive) individual weeds caused by the later herbicide application in GMHT crops (Heard et al., 2003b) tends to reduce the persistence of plant populations in the farmed landscape and reduce seed densities and in turn emerged plants. This loss of food resources is likely to cause reductions in the abundance of key invertebrate groups (Hawes et al., 2003) and of species at higher trophic levels, such as farmland birds. Predicting future changes in the timing of herbicide applications as had already occurred in the USA, where uptake of GMHT crops was driven by the perceived profitability of cropping, Heard et al. (2005) noted that alterations to the frequency of high-density weed patches in the landscape could have important implications, if the spatial distribution of weeds across the landscape affects interactions with higher trophic levels. Farmland birds that forage extensively on weed seeds in winter, aggregating in direct response to their abundance, may be particularly affected. Changes to rotations themselves are also likely, and may have considerable effects (both beneficial or adverse ones). Indeed, biodiversity differences between crops were shown to be comparable to those between treatments in separate studies (Firbank et al., 2003b; Lutman et al., 2008). All of the factors above will vary from region to region, from Member State to Member State, from season to season, and from biodiversity component to biodiversity component. These factors depend not only on the nature of the particular receiving environment, but on weed pressure, soil type and climatic conditions. For these reasons, whilst meaningful conclusions can be drawn from general principles, the EFSA GMO Panel acknowledges that there are considerable challenges to making accurate predictions on the environmental consequences of the use of herbicides in GMHT cropping systems. Predictions from models would need to consider all the issues detailed above, over the full range of possible parameters that may be varied in the management of the GMHT crops, and the full range of receiving environments within Europe. The complex nature of all these dynamic effects is of course be modulated further by market forces and agricultural economics.

Large-scale experimentation to determine the impacts of all the herbicide programmes incorporating glyphosate that are likely to be adopted by farmers in the different farming regions of each Member State cultivating maize GA21 is deemed infeasible for reasons of practicability and cost (e.g., Perry et al., 2003; Squire et al., 2003; Qi et al. 2008). Therefore, modelling may be attempted (e.g., Holst et al., 2007; Caron-Lormier et al., 2009, 2011), particularly to assess regional-scale (Firbank et al., 2003a) and long-term effects (Lutman et al., 2008) of possible changes in agricultural practice over the course of many rotations. However, present models do not provide a robust means of predicting outcomes, because of their critical dependence on underlying assumptions. Different models of the same system may give very different predictions and therefore caution must be exercised in reviewing the output of models. As an illustration, consider four models that were built around the GMHT cropping systems studied in the FSEs. In an initial assessment, Heard et al. (2003a,b) used long-term data from the decline in UK weed soil seedbanks and compounded this with the reduction in soil seedbank density found for dicotyledons in GMHT crops *other than maize* (i.e., for beet and oilseed rape). They predicted a worst-case decline in soil seedbanks of 7 % per annum for a 5-course cereal rotation with a break crop grown every five years. By contrast, they believed that it was quite possible that, under rotations including glufosinate-ammonium tolerant maize, weed populations would in the long-term be stable or increase. Heard et al. (2005) later revised and refined their earlier opinion for GMHT beet and oilseed rape, after taking into account density dependence of the weeds that integrated both population dynamics and grower response to weeds, within a 7-course, 4-year rotational framework. Gibbons et al. (2006) calculated the quantitative effects of changes in seed rain on the dietary requirements of 17 granivorous farmland bird species, although they declined to predict effects on individual bird species. They concluded that should beet, spring and winter oilseed rape in the UK be largely replaced by GMHT crops and managed as in the FSEs, this would markedly reduce important food resources for farmland birds, many of which had already suffered decline during the last 30 years. By contrast, glufosinate-ammonium tolerant maize would be beneficial to farmland birds. Butler et al. (2007) used a semi-qualitative approach and concluded that of 39 susceptible farmland bird species, even under nationwide introduction of the GMHT beet and oilseed rape systems studied in the FSE regimes, only one species would be re-classified to a less favourable conservation status due to the implementation of such systems. Grower uptake was predicted to have only a limited effect on Farmland Bird Indices. Further guidance on the need to upscale experimental results spatially and temporally, from field and season scales to region and decadal, multi-rotational scales was given by EFSA (EFSA, 2008b; see also Castellazzi et al., 2007, 2008).

Evidence indicates that the response of arthropods to altered weed abundance and composition is variable, being dependent on life-history characteristics (Brooks et al., 2003). The lower density of arable weeds on maize plots treated with glyphosate does not necessarily alter the biological control functions provided by natural enemies or lead to more insect pests (Albajes et al., 2008, 2009, 2011). This can be attributed to the complexity of arable ecosystems in which changes in arthropod composition may be influenced by the effect of functional redundancy in the system (Johnson, 2000), the crop itself (if it provides resources for arthropods), arthropod dispersal (Haughton and Bohan, 2008; Smith et al., 2008b), habitat heterogeneity (Benton et al., 2003) and interactions between habitat structure, land use and arthropod species ecology (Haenke et al., 2009; Goulson et al., 2010). In their paper, Albajes et al. (2009) reported that leafhoppers and aphids were more abundant in herbicide-treated plots, whereas phytophagous thrips were less abundant. Among predators, *Orius* spp., spiders, and trombidids were more abundant on treated plots, whereas nabids and carabids were more abundant in untreated plots; the same case was found for carabids and spiders caught in pitfall traps. Among parasitoids, ichneumonids were more abundant in untreated plots and mymarids in treated plots. The higher abundance of on-crop plant predators such as *Orius* spp. in treated plots was the result of more prey (e.g., leafhoppers and to a lesser extent aphids) in less-weedy maize fields. For *Nabis* sp., which was more abundant on untreated plots, no relation to any of the herbivores tested was shown (Albajes et al., 2011). In a continuation of the study by Albajes et al. (2009), where the impact of glyphosate-based herbicide regimes on non-target arthropods through the food web was compared with that of currently applied herbicide regimes, it was observed that populations of arthropod herbivores and natural enemies are not greatly affected, unless weed abundance is drastically altered (Albajes et al., 2010, 2011). This indicates that differences in weed abundance, induced by the adoption of different herbicide regimes, are not necessarily ecologically relevant (in terms of functionality) (e.g., Bohan et al., 2007; Smith et al., 2008b).

The EFSA GMO Panel concludes that indirect effects associated with the use of the complementary glyphosate-based herbicide regimes have the potential to cause adverse impacts on farmland biodiversity. The magnitude of this reduction in farmland biodiversity is dependent upon a series of factors (Table 2), which include the efficacy of the applied herbicide regimes in controlling weeds, crop rotations, and the level of farmland biodiversity sustained in receiving environments. In particular, the repeated use of glyphosate-based herbicides at recommended application rates on continuous maize GA21 may result in reductions in botanical diversity and/or weed density in maize fields to a level that might adversely affect food chains and webs, but not necessarily biological control functions, at the field and landscape level (Table 2). Such a reduction in biodiversity may be considered problematic by risk managers depending upon protection goals pertaining to their region, especially in receiving environments that sustain little farmland biodiversity or in environmentally sensitive areas.

Table 2. Major factors affecting the risk of reducing in-field botanical diversity based on expert judgment and historical experience

Management option	Risk of reducing in-field botanical diversity		
	Low	Moderate	High
Crop rotation	> four years, presence of functionally distinct crops (e.g., cereals/industrial crops/pulses) and seasonally distinct crops (winter vs. spring-summer)	Limited duration (two/three to four years) with reduced presence of functionally distinct and/or seasonally distinct crops	No rotation (continuous cropping)
Tillage system	Alternation between ploughing and minimum/no-till systems	Only minimum tillage or no-till	Only ploughing
Weed management in cropping system	Cultural, mechanical and chemical	Cultural and chemical, or mechanical and chemical	Mainly chemical
Use of same mode of action per season	Once	More than once	Many times
Landscape features (other regionally relevant factors)	Highly mixed crops on many small fields	Moderately mixed crops on medium-sized fields	Mostly one type of crop on large fields
Conservation headlands and/or uncultivated field margins	Presence	Limited presence	No presence

The EFSA GMO Panel conclusion on potential impacts of specific cultivation, management and harvesting techniques associated with the cultivation of maize GA21 is consistent with that of the CZ CA. In its evaluation, the CZ CA identified potential adverse effects of the herbicide used on maize GA21 on the environment, and they considered that "*along with increasing acreage where GA21 maize is to be cultivated a broader herbicide usage implications on the environment might be expected, which means that NTOs* [non-target organisms] *may be affected*" (section 8.1 of the environmental risk assessment report of the CZ CA).

Weed shifts and the selection of weed communities composed of more tolerant or resistant species

The sole usage of a single herbicide over a wide cropping area for an extended period is known to potentially cause changes in weed flora, and to increase the selection of communities dominated by tolerant weed species or of resistant weed biotypes (Gressel, 2009; Dewar, 2010; Reddy and Norsworthy, 2010; Owen, 2011; Green and Owen, 2011). There is evidence from cultivation of GMHT crops that the repeated, continuous and exclusive use of glyphosate in no- or reduced-tillage systems causes changes in weed flora, and favours the selection of more tolerant or resistant weed communities (Fernandez-Cornejo et al., 2002; Tingle and Chandler, 2004; Johnson et al., 2009; Kruger et al., 2009; Powles, 2008, 2010; Gressel, 2009; NRC, 2010; Owen et al., 2010, 2011; Powles and Yu, 2010; Waltz, 2010; Webster and Sosnoskie, 2010; Beckie, 2011; Heap, 2011; Shaner et al., 2011). The lack of residual activity of glyphosate may result in two to four applications of this herbicidal active substance per growing season, depending on weed seedling emergence patterns. In addition, no- or reduced-tillage systems, enabled by the use of glyphosate (Locke et al., 2008; Givens et al., 2009b), may further increase the selection pressure on weeds and weed density (Cardina et al., 2002). Because mechanical pre-plant weed control is reduced or completely replaced by the use of glyphosate in no- or reduced-tillage systems, herbicide applications, particularly pre-sowing applications, become more important (Givens et al., 2009a). Moreover, no- or reduced tillage systems concentrate weed seeds close to the soil surface (Bàrberi and Lo Cascio, 2001; Moonen and Bàrberi, 2004; Vasileiadis et al., 2007) from which they can more easily emerge, giving rise to increased in-field weed densities, which require more frequent glyphosate applications.

The increased selection pressure imparted by glyphosate may cause changes in abundance of selected weed populations or in species relative abundances (and consequently in weed community diversity). Weed shifts occur because of differential natural tolerance of glyphosate between species in a weed community or because of the spread of herbicide resistant biotypes (Norsworthy et al., 2001; Soukup et al., 2008; Reddy and Norsworthy, 2010). Glyphosate avoidance (non-exposure) is achieved either by very early weed emergence and rapid maturation, or by late season weed emergence. Weeds emerging after a glyphosate application can fill niches vacated by the weeds that were effectively controlled by glyphosate. Moreover, elimination of competition from early-season weeds create a favourable environment for late-season weeds (Owen, 2008; Reddy and Norsworthy, 2010). A survey of twelve weed scientists from eleven states across the USA, to assess weed shifts in GMHT maize, cotton and soybean, revealed that no weed shifts were observed in GMHR maize yet, and that this was attributed to the low adoption of GMHR maize (Culpepper, 2006). In a 6-year field study, Verschwele and Mülleder (2008) considered potential changes in weed communities due to the use of glyphosate in a continuous maize NK603 rotation at three sites in Germany, and did not observe statistically significant differences between local standard herbicide treatments and the glyphosate-based treatments on the mean values of seedbank, species richness, species diversity and dominance (see also Verschwele, 2011). The variation in weed seedbank size and composition was mainly attributed to site and year effects. While Verschwele and Mülleder (2008) showed that risks for weed population shifts from GMHT crops are no greater than those associated with other herbicides and non-GMHT crops, other studies reported or predicted shifts in weed populations due the increased frequency and rate of glyphosate use in GMHT crops (e.g., Shaner, 2000; Hilgenfeld et al., 2004; Duke, 2005; Owen and Zelaya, 2005; Puricelli and Tuesca, 2005; Culpepper, 2006; Scursoni et al., 2007; Owen, 2000, 2008). In case of GMHT maize, Wilson et al. (2007) found that over a 6-year period in glyphosate-based cropping systems in western USA corn belt, weed populations shifted from a kochia (*Kochia scoparia*) and wild-proso millet (*Panicum miliaceum*) dominated population to predominantly narrowleaf lambsquarters (*Chenopodium desiccatum*) population. Weed shifts may exacerbate weed problems and reduce the effectiveness of weed control (Young, 2006). With the anticipated increase in adoption of GMHR maize as well as application rates of glyphosate, more frequent weed shifts have been predicted (Shaner, 2000).

The use of glyphosate and its potential effects on the environment are also assessed under Regulation (EC) No 1107/2009. Questions related to the evolution of herbicide resistance to glyphosate in weeds are addressed by each Member State on receipt of the biological assessment dossier contained within the chemical market registration dossier. As part of the biological assessment dossier, applicants assess the likelihood of weed resistance evolving as a result of the use of glyphosate on GMHT crops, and provide a weed resistance management plan to delay this process[59]. The assessment of the likelihood of weed resistance evolving is in line with the European guidelines PP 1/213 of the European and Mediterranean Plant Protection Organization (EPPO, 2003). These guidelines propose a resistance risk analysis of two-stages, composed of resistance risk assessment, in which the probability of evolution of resistance and its likely impact are evaluated, and resistance risk management where, if necessary, possible strategies for avoiding or delaying the appearance of resistance are considered and suitable conditions of use are chosen and implemented. In resistance risk assessment, the inherent risk is first assessed using the characteristics of the pest and the product; the unmodified risk is then evaluated from the inherent risk when the product is applied under unrestricted conditions of use. In resistance risk management, the decision is made whether the unmodified risk is acceptable; if it is, the process can stop. If the unmodified risk is not acceptable, possible modifiers are then analysed to determine whether they can be used to mitigate the risk. If suitable modifiers exist, the conclusion of

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the resistance risk analysis will be a resistance management strategy (comprising one or more modifiers) that can be applied when the product is used commercially (EPPO, 2003).

Despite the low inherent risk of resistance evolution in weed species attributed to the biochemical, chemical and biological properties of glyphosate in plants and soil (Bradshaw et al., 1997), instances of weeds evolving resistance to glyphosate under field situations have been reported since 1996 (e.g., Powles et al., 1998; Pratley et al., 1999). Since then, there have been increasing instances of evolved glyphosate resistance in some weed species, especially following the advent of GMHT crop cultivations (Owen and Zelaya, 2005; Sanderman, 2006; Powles, 2008; Beckie, 2011; Heap, 2011; Green and Owen, 2011; Owen et al., 2011), contradicting the initial speculations that the evolution of glyphosate resistant weeds was unlikely (Bradshaw et al., 1997). Currently, 21 weed species have evolved glyphosate resistant populations globally and twelve glyphosate resistant weed species have been identified in the USA, most of which evolved resistance to glyphosate in GMHT cropping systems (Beckie, 2011; Heap, 2011). The basis for resistance has been attributed to altered EPSPS target site, reduced translocation or cellular transport to the symplast, and sequestration in the vacuole (reviewed by Powles, 2008; Powles and Yu, 2010; Beckie, 2011; Shaner et al., 2011; Vila-Aiub et al., 2011). The problem of glyphosate resistant weeds is exacerbated by the fact that new resistance mechanisms such as gene amplification are being found (i.e., Gaines et al., 2010). Moreover, the evolution of multiple and cross resistances to herbicides is becoming increasingly more common (Heap, 2011). The overreliance on glyphosate to control herbicide resistant weeds contributed to the evolution of multiple resistances in populations (i.e., two or more resistance mechanisms) as a consequence of sequential selection or pollen flow, such as in glyphosate resistant *Lolium* spp. in Australia and South Africa (Neve et al., 2004; Yu et al., 2007; Preston et al., 2009; Preston, 2010) and in *A. palmeri* in cotton fields in southern USA (Culpepper et al., 2010). Multiple resistances to ALS-inhibiting herbicides and glyphosate are reported in horseweed (*Conyza canadensis*) (Davis et al., 2009).

It is important to note that glyphosate does not ‘cause’ weeds to evolve resistance *per se*, but rather how it is used that leads weeds to evolve resistance (Owen et al., 2011; Wilson et al., 2011). Evidence from the USA confirms that, where there is very intense glyphosate selection (i.e., glyphosate tolerant maize monocultures or glyphosate tolerant maize-soybean rotations), little diversity in weed control practices and no mandated herbicide resistance programmes (Waltz et al., 2010), glyphosate resistant weeds may evolve and spread rapidly (e.g., Dauer et al., 2009; Owen et al., 2011). This in turn may induce modification of farmers’ weed management practices through intensification of herbicide usage and subsequent adverse environmental effects (Johnson et al., 2009; Kruger et al., 2009; Shaw et al., 2009; Webster and Sosnovski, 2010). In regions where glyphosate resistant weeds have to be controlled, farmers might exacerbate this phenomenon by increasing rates of glyphosate applied, which may further increase the selection pressure on weeds and lead to more instances of resistance (Duke, 2005; Pline-Srnic, 2005; Neve, 2008; Owen et al., 2011).

While the scale of glyphosate resistant weed outbreaks has remained relatively small so far, a concern is that glyphosate resistant weeds would become more widespread in the near future (Service, 2007), as this would represent a significant threat to the sustainability of the herbicide and trait, and perhaps to global food production (Duke and Powles, 2008a; Powles, 2010; Owen et al., 2011; Ronald, 2011).

The EFSA GMO Panel concludes that the cultivation of maize GA21 in monoculture or in rotation with other glyphosate tolerant crops, in conjunction with the repeated and/or exclusive application of glyphosate-based herbicides will cause changes in weed flora, and will favour the evolution and spread of glyphosate resistant weeds due to the selection pressure exerted by glyphosate. This, in turn, may affect food webs, and the functional value of weed vegetation for organisms of higher trophic levels (reduced functional biodiversity). However, where there is more diversity in weed control practices and crop rotation, and where mandated herbicide resistance programmes are put in place, the selection pressure of glyphosate on weeds will be reduced, decreasing the selection of more tolerant or resistant weeds significantly. In general, those management options that lead to a high risk of reduction of botanical biodiversity (Table 2) also favour resistance evolution.

The EFSA GMO Panel conclusion on potential impacts of specific cultivation, management and harvesting techniques associated with the cultivation of maize GA21 is consistent with that of the CZ CA. In its evaluation, the CZ CA considered that "*the widespread use of a single herbicide product could potentially lead to the selection of increased resistance within species that were previously susceptible. Furthermore, a potential risk for weed spectrum shifts associated to herbicide crop management could arise*" (section 8.1 of the environmental risk assessment report of the CZ CA).

Impact on soil microbial communities

While no direct adverse effects of the mEPSPS protein have been reported on non-target organisms, biogeochemical processes and the abiotic environment (sections 6.2.4.2 and 6.2.6), the herbicide management associated with the cultivation of maize GA21 may under certain circumstances have adverse effects on the biotic environment and biogeochemical processes. As for glyphosate susceptible plants, the application of glyphosate can inhibit the EPSPS protein of some microorganisms and thus decrease or fully inhibit the synthesis of aromatic amino acids (Busse et al., 2001; Zablotowicz and Reddy, 2004). The entry of glyphosate into soil may affect the soil microbial community directly, by providing energy, carbon, nitrogen or phosphorus sources, or by inhibiting growth of glyphosate susceptible bacteria and fungi. Consequently, transient shifts in microbial biomass and/or the structural diversity of the microbial community may occur. Overall, the addition of glyphosate as a technical compound or in it commercial product Roundup Ultra results in increased microbial biomass accompanied by higher soil respiratory activity, giving no rise to conclude on adverse effects on microbial communities (Haney et al., 2000).

Evidence suggests that glyphosate can affect soil and rhizosphere-inhabiting bacteria and fungi, including those capable to live in mycorrhizal relationship, and rhizobia, the latter group is characterised by its capacity to establish symbiosis with specific legumes by mediating nitrogen fixation in root nodules (Busse et al., 2001; Zablotowicz and Reddy, 2004, 2007; Means et al., 2007; Kremer and Means, 2009; Powell et al., 2009a). Potential consequences of frequent glyphosate applications in GMHT cropping systems comprise alterations in the microbial ecology and biological processes carried out in the crop rhizosphere, and may encompass effects on potential phytopathogen antagonist interactions, and interference with plant-growth-promoting rhizobacteria (Lupwayi et al., 2009). Impacts of glyphosate on microbial communities however are thought to be limited, because the majority of soil microorganisms appear not to be affected by glyphosate due to high tolerance to glyphosate and the instability of the herbicide in soil, which decreases exposure. In fact, a proportion of glyphosate is sorbed by soil particles where its activity is limited, while free (water-dissolved) glyphosate is degraded by soil microorganisms (Haney et al., 2002; Powell et al., 2009a). It should be noted that sorbed compounds are in equilibrium with their water-dissolved molecules including sorbed glyphosate and thus also expected to decline. Glyphosate may however transiently accumulate. The entry of glyphosate into soil may not only occur directly by spraying of the herbicide, but also indirectly through plant roots of GMHT plants since plants do not metabolize glyphosate but translocate it to actively growing regions, including roots, from which it can then enter the soil. The exudation of glyphosate would cause direct exposure in the rhizosphere where beneficial plant growth promoting microorganisms, including myorrhiza and nitrogen-fixing symbiotic bacteria, compete with potential plant pathogens (Feng et al., 2005; Kremer et al., 2005). Potentially this may generate a bias to the disadvantage of the plant (Powell and Swanton, 2008).

In their field experiment, Liphadzi et al. (2005) found that the use of glyphosate on glyphosate tolerant maize did not affect soil respiration and the diversity of the dominant soil bacteria. Likewise, it was found that neither the diversity of denitrifying bacteria nor that of root-colonizing fungi was affected by glyphosate applied on glyphosate tolerant maize (event DKC3551) (Hart et al., 2009). Weaver et al. (2007) did not observe significant effects of glyphosate on soil microbial communities and its mineralisation in bulk soil or rhizosphere soils, even at concentrations well above recommended field application rates. Barriuso et al. (2011) showed that glyphosate treatments applied on GMHT maize during the three-year period of seasonal cultivation in two different fields did not significantly change the maize rhizobacterial communities when compared to those of the untreated soil. However, the repeated application of glyphosate at a rate of 49 µg ai g^{-1} soil to the soil surface, representing the concentration of glyphosate present following a field application in a 2 mm soil interaction depth, was shown to induce shifts in soil microbial communities (Lancaster et al., 2009). These and other studies indicate that the single application of glyphosate has only small and transient effects on the soil microbial community (Motavalli et al., 2004; Gomez et al., 2009; Barriuso et al., 2010, 2011), if they occur, and that its repeated use may favour those soil microorganisms capable of metabolising glyphosate or tolerant to the herbicide (Lancaster et al., 2009). It should be noted that such temporal responses of the soil microbial biomass to the addition of glyphosate are not unusual when microbiological substrates are added to soil. To the knowledge of the EFSA GMO Panel, a persisting effect of glyphosate on soil microbial communities has not been reported.

Studies on glyphosate interactions with microbial communities in GMHT cropping systems revealed that the most pronounced effects are detected with specific groups (genera or species) of microorganisms (such as fungi of the genera *Fusarium* and *Pythium* or bacteria from the Pseudomonas group) rather than with broader measurements of soil microbial diversity and functions (Kowalchuk et al., 2003; Lupwayi et al., 2007; Means et al., 2007; Powell and Swanton, 2008; Hart et al., 2009a). These microorganisms are often typical inhabitants of crop rhizospheres. Responses of individual fungal species varied depending on their susceptibility to glyphosate; some species express glyphosate-sensitive forms of EPSPS and may not metabolise glyphosate (Morjan et al., 2002). In a laboratory study, growth of *Pythium ultimum* and *F. solani* could be stimulated or inhibited, depending on glyphosate concentration (Kawate et al., 1992). Kremer and Means (2009) observed that *Fusarium* spp. colonisation levels of roots of glyphosate tolerant maize receiving glyphosate were three to ten times higher than those for the atrazine treatment, indicating that glyphosate induces fungal colonisation of maize rhizospheres and hence affects the ability of plants to suppress potential pathogen colonisation and root infection. Such effects have also been observed under controlled conditions, but in a recent review, Powell and Swanton (2008) argued that experimental field trials, investigating the link between glyphosate and crop diseases associated with *Fusarium* spp., are not representative of interactions that occur under actual farming conditions. Moreover, not all *Fusarium* strains respond in the same way; some strains did not grow better in glyphosate-based plant exudates (Kremer et al., 2005). A negative relationship between the population size of culturable fluorescent pseudomonads and root colonisation by *Fusarium* spp. was shown in glyphosate tolerant soybean (Kremer and Means, 2009). Fluorescent pseudomonads include some important plant growth promoting bacteria capable of producing relevant secondary metabolites in the rhizosphere, and they may transiently be reduced in glyphosate tolerant soybean due to their reported sensitivity to glyphosate (Zobiole et al., 2011). Glyphosate may enhance fungal root colonisation and potential diseases by stimulating growth of the fungal pathogen and by suppressing bacterial antagonists (Powell and Swanton, 2008). Powell et al. (2009b) have also reported that, depending upon the location of litter placement, glyphosate use can significantly reduce maize litter decomposition.

In soybean, glyphosate has been shown to be preferentially translocated metabolically active, growing plant compartments, including nodules where nitrogen-fixing symbionts (bacteroids) of the bacterial species *Bradyrhizobium japonicum*, which possess a glyphosate sensitive EPSPS protein, can be exposed to it. Upon exposure to glyphosate, bacteroids may accumulate high concentrations of shikimate and certain benzoic acids that could inhibit plant growth. These effects are accompanied by growth inhibition and/or death of bacteroids, depending upon the glyphosate concentration (Cerdeira and Duke, 2006). Zablotowicz and Reddy (2004, 2007) also reported that glyphosate affects root nodulation and nitrogen fixation of Bradyrhizobium compared on conventional soybean in contrast to other herbicides. The consequences of this could be that glyphosate applications may reduce, probably transiently, populations of Bradyrhizobium or other root-nodule forming rhizobia, and thus reduce the natural potential to form symbiosis with nitrogen-fixing legumes (Reddy et al., 2000; King et al., 2001; Reddy and Zablotowicz, 2003; Bohm et al., 2009; Zobiole et al., 2010). In cropping systems, this could reduce the nodulation of the crop and thus increase the need for additional nitrogen fertilisers in nitrogen-depleted soil (Bohm et al., 2009). Agricultural practice yet shows that nodulation of host-plants by rhizobia can be inconsistent, varying unpredictably with the rate and timing of the glyphosate application (Cerdeira and Duke, 2006; Powell et al., 2009a). It was even observed that nitrogen fixation was greater for glyphosate tolerant soybean treated with glyphosate than for untreated plants, but only when glyphosate was applied at the first trifoliate soybean growth stage (Powell et al., 2009a). Nitrogen-fixing Bradyrhizobium or other rhizobia have no importance for maize, as it is not nodulated by these bacteria which are specific for legumes (peas, soybean, etc.). Beside symbiotic nitrogen-fixing rhizobia of legumes, there are several common soil inhabiting bacteria with a potential to fix nitrogen without root nodule formation. Such bacteria may reside in the intracellular space in roots or shoots or in the rhizosphere, but their contribution for supplying the respective crops with nitrogen is under normal agricultural conditions generally very low.

Whilst glyphosate can affect soil bacteria, mycorrhizal fungi and Bradyrhizobium or other rhizobia, such effects have been observed inconsistently, and mostly in other glyphosate tolerant crops than maize. Rearrangements in structural diversity and population abundance of soil microbial communities occur frequently in the agricultural environment. They are typically associated with several sources of variation, caused by natural variability (e.g., soil heterogeneity, weather conditions) and agricultural practices (e.g., soil tillage, crop rotation, irrigation measures) and are thus not necessarily an indication of environmental harm (Kowalchuk et al., 2003). The magnitude and direction of responses of the soil microbial community to glyphosate application depend on herbicide dose, soil and microorganisms investigated (Gorlach-Lira et al., 1997; Motavalli et al., 2004), and ecological interactions, including whether studies are conducted under laboratory or field conditions (Wardle and Parkinson, 1990, 1992; Busse et al., 2001; Motavalli et al., 2004; Powell and Swanton, 2008; Savin et al., 2009). The EFSA GMO Panel considers that potential effects on soil microbial communities and the ecological functions they provide, due to the use of glyphosate on maize GA21 at normal field application rates, if they occur, will be transient and minor, and are likely to be smaller or within the range currently caused by other agronomic and environmental factors. Therefore, the EFSA GMO Panel concludes that the use of glyphosate-based herbicides on maize GA21 is unlikely to cause adverse effects to soil microbial communities or beneficial functions mediated by them at normal field application rates. The EFSA GMO Panel notes that effects of herbicidal active substances on soil microbial communities are considered through functional tests on nitrification and soil respiration under Regulation (EC) No 1107/2009.

In its evaluation, the CZ CA considered that "*that glyphosate might have effects on soil microbial communities as reported for other crops and thus reduce microbial functions and contributions to field ecosystems. For example, applications of glyphosate may result in reduction of rhizobial population. On the other hand, it should be noted that only a small percentage of the microorganisms related to the plant rhizobial communities can be grown under laboratory conditions (Torsvik et al., 1990) and therefore the composition of these microbial communities is largely unknown. Nevertheless, it is fair to assume that the evolution of these communities ought to be affected by the extensive use of glyphosate*" (section 7.5 of the environmental risk assessment report of the CZ CA).

6.2.8. Conclusion on the environmental risk assessment

Since the scope of the current application covers cultivation, the environmental risk assessment considered the environmental impact of full-scale commercialisation of maize GA21.

The CZ CA (including its Biosafety Commission) provided to EFSA its report on the environmental risk assessment of maize GA21 (dated 20 October 2010) on 25 October 2010 in line with Articles 6.3(c) and 18.3(c) of Regulation (EC) No 1829/2003. The report on the environmental risk assessment of the CZ CA is provided in Annex H of the EFSA Overall Opinion, and has been considered throughout this EFSA GMO Panel Scientific Opinion.

The EFSA GMO Panel considers that maize GA21 has no altered agronomic and phenotypic characteristics, except for the herbicide tolerance. The likelihood of unintended environmental effects due to the establishment, survival and spread of maize GA21 is considered to be extremely low, and will be no different from that of conventional maize varieties.

It is highly unlikely that the recombinant DNA will transfer and establish in the genome of bacteria in the environment or human and animal digestive tracts. In the rare but theoretically possible case of transfer of the m*epsps* gene from maize GA21 to soil bacteria, no novel property would be introduced into the soil bacterial community and thus no positive selective advantage that would not have been conferred by natural gene transfer between bacteria would be provided.

Based on the evidence provided by the applicant and relevant scientific literature on maize GA21, the EFSA GMO Panel concludes that there are no indications of adverse effects on non-target organisms due to unintended changes in maize GA21, and therefore considers *trait*-specific information appropriate to assess whether maize GA21 poses a risk to non-target organisms.

The studies, supplied or reviewed by the applicant, showed no adverse effects on different types of non-target organisms due to the expression of the mEPSPS protein in glyphosate tolerant crops.

The EFSA GMO Panel does not expect potential adverse effects on biogeochemical processes and the abiotic environment due to the expression of the mEPSPS protein in maize GA21.

The EFSA GMO Panel is of the opinion that potential adverse environmental effects of the cultivation of maize GA21 are associated with the use of the complementary glyphosate-based herbicide regimes. These potential adverse environmental effects comprise (1) a reduction in farmland biodiversity, (2) changes in botanical diversity due to weed shifts, with the selection of weed communities mostly composed of tolerant species, and (3) the selection of glyphosate resistant weeds. The potential harmful effects could occur at the level of arable weeds, farmland biodiversity, food webs and the ecological functions they provide. The magnitude of these potential adverse environmental effects will depend upon a series of factors, including the specific herbicide and cultivation management applied at the farm level, the crop rotation and the characteristics of receiving environments.

The EFSA GMO Panel considers that the use of glyphosate-based herbicides at recommended field application rates of glyphosate on maize GA21 is unlikely to cause adverse effects to soil microbial communities or beneficial functions mediated by them.

The conclusions of the EFSA GMO Panel on the environmental safety of maize GA21 are consistent with those of the CZ CA. The CZ CA concluded that "*based on the existing information and data provided by the Syngenta Company within the evaluation process, the Czech CA considers that maize GA21 has no altered survival, multiplication or dissemination characteristics and interacts with other organisms as any conventional maize. However, the data presented on the issue of "Impacts of the specific cultivation, management and harvesting techniques" do not allow a comprehensive assessment of potential long-term effects on the environment associated to the use of the herbicide*" (section 9 of the environmental risk assessment report of the CZ CA). Hence, the CZ CA identified "*no potential effects on the environment either immediate, delayed, direct or indirect with the exception of those related to the change in the herbicide management*" (section 8.1 of the environmental risk assessment report of the CZ CA).

6.3. Risk management strategies (including post-market environmental monitoring)

6.3.1. Risk mitigation measures

6.3.1.1. General aspects of mitigation

According to the EFSA GMO Panel guidelines on the environmental risk assessment of GM plants (EFSA, 2010e) and in line with Annex II of the Directive 2001/18/EC, the risk assessment can identify risks that require management and propose risk mitigation measures to reduce the levels of risk. In order to reduce the identified risks associated with the GM plant deployment to a level of no concern, both the CZ CA and the EFSA GMO Panel evaluated the scientific quality of the risk mitigation measures proposed by the applicant, as well as their adequacy and efficacy. Risk mitigation should be proportionate to the results of the different risk scenarios studied, the specific protection goals in the receiving environments, and to the levels of scientific uncertainty and risk identified in the environmental risk assessment (EFSA, 2011c).

6.3.1.2. Interplay between environmental risk assessment and mitigation

The environmental risk assessment of maize GA21 concluded that maize GA21 plants are unlikely to cause any direct adverse effects, but that the cultivation of maize GA21 may result in adverse environmental effects due to the use of the complementary glyphosate-based herbicides. These potential adverse environmental effects comprise (1) a reduction in farmland biodiversity, (2) changes in botanical diversity due to weed shifts, with the selection of weed communities mostly composed of tolerant species, and (3) the selection of glyphosate resistant weeds. As the magnitude of these potential adverse environmental effects will depend upon a series of factors, the EFSA GMO Panel recommends that risk mitigation measures are put in place to ensure that glyphosate on maize GA21 will be used in diversified cropping regimes that have similar or reduced environmental impacts compared with conventional maize cultivation.

The specific risks identified in section 6.2.8 (conclusion on the environmental risk assessment), requiring mitigation, are (1) a reduction in farmland biodiversity due to novel herbicide regimes, (2) changes in botanical diversity due to weed shifts, with the selection of weed communities mostly composed of tolerant species due to novel herbicide regimes, and (3) the selection of glyphosate resistant weeds due to novel herbicide regimes. The EFSA GMO Panel notes that for these risks the possible environmental effects are related to the use of the complementary herbicide, and judges that risk mitigation measures could equally well be put in place either under the legislation for plant protection products (Regulation (EC) No 1107/2009, which replaced Directive 91/414/EEC on 14 June 2011, and Directive 2009/128/EC), or under the legislation for GMOs (Directive 2001/18/EC). In reaching this view, the EFSA GMO Panel considered: the interplay between the legislation for GMOs and plant protection products (section 6.2.7.1); the fact that some herbicide tolerant systems on the market are non-GM; and the fact that protection goals are set at Member State level. However, since the remit of the EFSA GMO Panel to propose risk mitigation measures is linked inextricably to Directive 2001/18/EC, subsequent recommendations in this section are based on GMO legislation.

Possible risk mitigation measures, which can be put in place to reduce levels of risk and remaining scientific uncertainty, and their efficacy, were evaluated by the EFSA GMO Panel, and this evaluation is described below.

6.3.1.3. Risk mitigation measures to reduce adverse effects due to the use of novel herbicide regimes

The applicant proposed that "*a Technical Guide will be developed for farmers in order to ensure the implementation of good agricultural practices, covering recommendations on minimum and maximum application dose rates, herbicide mixtures and herbicide rotation in cropping systems*"[60].

Depending upon protection goals set at Member State level (e.g., EFSA, 2010c,d,e) and in situations where potential adverse herbicide effects are likely, risk managers should consider putting risk mitigation measures in place to manage potential herbicide effects and to ensure the implementation of good agricultural practices, including integrated pest management. Such measures should ensure that biodiversity is maintained at current levels, and that potential adverse effects on arable weeds, farmland biodiversity, food webs and the ecological functions they provide are limited to the levels currently found in non-GMHT maize. Likewise, whenever relevant, risk managers should recommend putting specific risk mitigation measures in place to reduce the selection of more tolerant or resistant weeds.

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[60] Additional information received on 13 July 2009 / Question 3 / Pages 8-13 / Appendix 9 // Additional information received on 17 February 2010 / Question 6 / Pages 22-27 / Appendix 7

Impact on farmland biodiversity

In line with protection goals set at Member State level by relevant legislations and according to the legal provisions of Directive 2001/18/EC (e.g., EFSA, 2010c,d,e), appropriate measures should be put in place to mitigate potential environmental adverse herbicide effects on biodiversity by targeting their main drivers (Butler et al., 2007). Member States may recommend using glyphosate on maize GA21 only in regimes that have similar or reduced environmental impacts compared with conventional maize cultivation, and that do not interfere with biological functions currently supported by maize cropping systems. The EFSA GMO Panel also notes that the new legislations for the assessment and use of plant production products, introduced biodiversity more explicitly as a protection goal. Regulation (EC) No 1107/2009 mentions that plant protection products shall have no unacceptable effects on the environment, especially on biodiversity and the ecosystem, whereas the use of herbicides will have to adhere to the principles of integrated pest management and be consistent with good plant protection practice in order to ensure high levels of protection of human and animal health and the environment. In addition, Member States will describe in their national action plans how they ensure that the general principles of integrated pest management as set out in Annex III of Directive 2009/128/EC on the sustainable use of pesticides are implemented by all professional users by 1 January 2014.

The EFSA GMO Panel considers that the delivery of both food production and biodiversity conservation should be reconciled at the field and landscape level (Firbank, 2005; Benton, 2007; EFSA, 2008b; Sutherland et al., 2009; Godfray et al., 2010). Maize has been shown to be a poor crop for biodiversity under European conditions, having the greatest adverse effect on farmland biodiversity compared with oilseed rape and beet (Dewar et al., 2005; Firbank et al., 2005b; Bohan et al., 2007; Smith et al., 2008b). Moreover, maize is frequently not grown in rotation with other crops in the EU (FCEC, 2009), so the repeated use of glyphosate at recommended application rates on continuous maize GA21 may result in reductions in botanical diversity and/or weed density in maize fields to a level that might adversely affect food chains and webs. In addition, plant communities in cropped and uncropped areas of the farm differ; it is therefore questionable whether providing plant resources on uncropped land only will be sufficient to reverse the declining trends in farmland biodiversity. Beneficial weed species adapted to the cropped area of the field can be distinct from the flora found in uncropped land, so sustaining their populations would increase the overall functional diversity of the farm ecosystem (Storkey, 2006). Besides plant communities, also the scale of cropped and uncropped areas of the farm differs, with the uncropped land usually representing a small percentage of the total area of the farm. Furthermore, Roschewitz et al. (2005) established that plant species diversity in agricultural landscapes is not only affected by management of single fields, but also by the heterogeneity of the surrounding landscape. It also remains debatable whether increases in crop yield will spare land for biodiversity and hence natural habitats from conversion into arable land in European countries (i.e., Balmford et al., 2005; Mooney et al., 2005; Matson and Vitousek, 2006; Ewers et al., 2009; Godfray et al., 2010). Therefore, the EFSA GMO Panel recommends that risk mitigation measures are put in place that can provide considerable benefits for biodiversity at the cost of no or only small reductions in crop yield (Dewar et al., 2003; May et al., 2005; Pidgeon et al., 2007).

A number of options for risk mitigation measures are possible, and can be divided into those that target uncropped land such as field margins and set-aside, or cropped areas (Marshall and Moonen, 2002; Kleijn and Sutherland, 2003; Kleijn et al., 2006; Storkey and Westbury, 2007; Kleijn et al., 2011; Whittingham, 2011). Possible risk mitigation measures for uncropped land include protecting adjacent habitats from herbicide effects. To limit potential adverse effects due to herbicide drift, the approval for the application of glyphosate on Roundup Ready maize includes recommendations for separation distances of 20 m from certain sensitive areas and measures for the protection of water courses in Germany (Streloke, 2011). Impacts on biodiversity may also be mitigated by better field margin management or other ‘out of crop’ measures, which are increasingly applied in conventional cropping systems to deliver desired ecological benefits (Marshall, 1989; Wilson and Aebischer, 1995; Thomas and Marshall, 1999; Norris and Kogan, 2000; Marshall and Moonen, 2002; Meek et al., 2002; Roschewitz et al., 2005; Moonen et al., 2006; Butler et al., 2007; Clarke et al., 2007; Walker et al., 2007; Smith et al., 2008a; Dewar, 2009; Fried et al., 2009; Cordeau et al., 2011). Headlands and/or field margins, as being part of the field margin complex (Greaves and Marshall, 1987), are strips of land lying between crops and the field boundary, and extending for a limited distance into the crop (Marshall and Moonen, 2002). These margins fall into two broad categories (1) uncropped, either sown (with grass or grass and wildflower seed mixes) or left to regenerate naturally (including naturally regenerated or sown [temporary or long-term] set-aside margins), and (2) cropped, comprising sown arable crops usually under modified management, such as conservation headlands, wild bird cover crops and pollen and nectar mixes. Cropped and uncropped margins can be managed in a range of ways particularly in terms of cutting and/or cultivation (reviewed by Vickery et al., 2009). Sensitive management of field margins can increase species density in agro-ecosystems, provide habitats for rare or endangered species, and enhance ecosystem services (Marshall and Moonen, 2002; Moonen et al., 2006; Vickery et al., 2009). Conservation headlands allow less intensive management by reducing fertiliser and pesticide inputs to field edges and margins (Sotherton, 1991; Kleijn and Snoeijing, 1997; Kleijn and Van der Voort, 1997), and can be supplemented with unsprayed field margin strips or semi-permanent beetle banks (Thomas et al., 2001; Fried et al., 2009). Field margins can also include boundary features such as hedgerows and ditches which are an extremely valuable habitat for invertebrates and birds, providing food, shelter and nest cover (Jobin et al., 2001; Fuller et al., 2004; Pywell et al., 2005a,b). These conservation areas would help to maintain a relative high degree of weed diversity at field edges.

In cropped areas, delayed or less intense in-crop weed management can promote arable weed communities, and thereby deliver benefits for farmland biodiversity. Heard et al. (2005) believed that growers might learn to tolerate higher weed densities at certain periods of the growing cycle, provided that these weeds do not cause economic loss. For fodder beet treated with glyphosate, Strandberg and Pedersen (2002) reported that with careful management according to label recommendations or with further delays to applications, there may be significant improvements of weed flora and arthropod fauna, but that weed seed production was reduced. Less intense in-crop weed management with glyphosate applied to a proportion of the field or crop can also maintain desired levels of biodiversity. In GMHT sugar beet, this can be achieved either by over-the-row band spraying to allow early season weed growth between, but not within, crop rows, or by overall spraying early only to allow some later emerging weeds. Weeds occurring between rows after an early over-the-row band spraying could be controlled by a later overall spray (Dewar et al., 2000, 2003; May et al., 2005; Pidgeon et al., 2007). Results showed that some weeds can be left for a longer period between the crop rows without yield loss (Dewar et al., 2003). These weeds can increase the number of beneficial invertebrates during the early to mid-season (Dewar et al., 2003), and produce seed in the autumn as food for birds (May et al., 2005). Such risk mitigation measures can also be applied to glyphosate tolerant maize. A UK field study, conducted in conventional maize with glyphosate using shielded sprayers, revealed that an early over-the-row band spray followed by an overall spray enabled to maintain levels of farmland biodiversity in maize without yield loss (DEFRA, 2004). Another in-crop risk mitigation measure is to rotate crops. Bourassa et al. (2010) reported that rotating maize with oilseed rape had a stronger effect on carabid community than did changing from conventional to GMHT maize, indicating that GMHT maize has little impact on the overall carabid fauna, but that it may influence the activity of certain species through effects on the weed community (see also Bourassa et al., 2008). The choice of crop sequences can be an important tool for manipulating weed communities to select potentially against pernicious species (Squire et al., 2000; Anderson, 2009). Rotating maize with other crops would also be the best preventive strategy against the selection of more tolerant or resistant weeds (see below).

In-crop risk mitigation measures can be more difficult to implement than managing uncropped land and field margins for biodiversity (Squire et al., 2000; Hawes et al., 2010). Managing weeds within the crop to support biodiversity involves the risk of reducing crop yield (Oerke, 2006) and the long-term build-up of problem weed communities (Storkey, 2006); there is an inevitable challenge in maintaining effective control of problem weeds, while sustaining beneficial weed species at economically acceptable levels (Storkey, 2006; Storkey and Cussans, 2007). It has been argued that more robust tools are required for assessing beneficial weed communities in terms of the ecological functions they provide to the ecosystem and their effect on crop yield, and ultimately to identify the appropriate threshold level of these weeds that is economically acceptable and ecologically significant (i.e., Bastiaans et al., 2000; Storkey, 2006; Storkey and Cussans, 2007). Another difficulty is that protection goals are not always clearly defined, as reaching agreement on what needs to be protected from harm (i.e., protection goals) presents challenges (Nienstedt et al., 2011; Sanvido et al., 2011a, 2012). So far, risk managers have failed to define clearly 'how many weeds' or 'what type of weeds' are desired in arable fields (Sanvido et al., 2011b), hampering the choice of risk mitigation measures that are proportionate to the specific protection goals in the receiving environments.

In its evaluation, the CZ CA was of the opinion that "*measures should be put in place under Directive 91/414/EEC and consecutively under Regulation (EC) No 1107/2009 to ensure compliance with regulatory requirements for the pesticide regimes used in Member States. These should include measures for the appropriate management of glyphosate on GMHT maize in each Member State permitting the use of glyphosate on maize GA21*" (section 7.6.2 of the environmental risk assessment report of the CZ CA). The CZ CA recommended the applicant "*to design appropriately a relevant Technical User Guide for farmers that should involve Good Agricultural Practices of glyphosate applications guaranteeing sustainable and safe use of the entire GA21 technology*" (section 9 of the environmental risk assessment report of the CZ CA).

Weed shifts and the selection of weed communities composed of more tolerant or resistant species

Based on the specific biochemical, chemical and biological properties of glyphosate in plants and soil, the applicant argued that the inherent risk of weed resistance to glyphosate may be considered low to medium, depending upon the weed species[61]. Despite the low to medium inherent risk of weed resistance to glyphosate, tolerant and resistant weeds are evolving in countries with extensive and repeated use of glyphosate, especially on GMHT crops (e.g., USA and Argentina) (reviewed by Beckie, 2011; Heap, 2011; Owen, 2011). Current maize management in the EU differs from region to region depending on the levels of adoption of certain agricultural practices including crop rotations, mechanical weed control, herbicide mixtures and/or herbicide rotation in cropping system. However, in some parts of the EU, continuous maize is grown, so there is a potentially high risk if glyphosate is repeatedly used on glyphosate tolerant maize (section 6.2.7). Therefore, the applicant has stated that the use of herbicides with different modes of action and compliance to best practices, such as scouting for weeds and the use of crop rotations will be applied in line with the stewardship guidelines for herbicide labels made by the Herbicide Resistance Action Committee (HRAC) industry group[62].

The EFSA GMO Panel considers it essential that diversified systems are maintained, and agrees with the applicant that integrated weed management programmes that aim at improved diversity in crop management and weed control practices can enable the mitigation of weed shifts and can delay weed resistance evolution (reviewed by Beckie, 2011; Shaner et al., 2011). Such measures could be put in place under Regulation (EC) No 1107/2009 or Directive 2009/128/EC to ensure compliance with regulatory requirements, operating in Member States, for the use of plant protection products. Such measures could ensure the appropriate management of glyphosate on GMHT maize, so that the evolution of resistant weeds is delayed. Scientific evidence showed that the selection pressure on weeds can be reduced by crop rotation (i.e., rotating glyphosate tolerant crops with non-glyphosate tolerant crops), using variable application rates and timing, applying a variety of herbicidal active substances with different modes of action, and by using non-herbicide weed control tools such as post-emergence cultivation and cover crops (Gressel and Segel, 1990; Liebman and Dyck, 1993; Gardner et al., 1998; Doucet et al., 1999; Cardina et al., 2002; Neve et al., 2003a,b; Nazarko et al., 2005; Beckie et al., 2006; Culpepper, 2006; Sammons et al., 2007; Gustafson, 2008; Owen, 2008; Werth et al., 2008; Beckie and Reboud, 2009; Busi and Powles, 2009; Gressel, 2009; Gulden et al., 2009; Shaw et al., 2009; Meissle et al., 2010; NRC, 2010; Werth et al., 2010; Beckie, 2011; Owen et al., 2011; Wilson et al., 2011). Using combinations of different weed management practices in integrated and diverse systems will reduce the selection pressure of any single practice or product (Sammons et al., 2007; Green and Owen, 2011; Shaner et al., 2011).

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[61] Additional information received on 17 February 2010 / Question 6 / Pages 23-27 / Appendix 7

[62] Additional information received on 13 July 2009 / Question 2 / Pages 7-8

In contrast to monocultures, crop rotation allows alternative weed control strategies to be used that may reduce weed population densities and favour a more diverse composition of weed communities, instead of communities that are dominated by one or few weed species. It remains however difficult to isolate the effects of crop rotation on weed communities from the weed control strategies that are used for the production of the crop. In some studies, the cropping sequence has been reported to be the dominant factor affecting the weed soil seedbank (Cardina et al., 2002), whereas, in others, crop rotation did not affect weed densities (e.g., Ball, 1992; Doucet et al., 1999). Moreover, crop rotation and weed control strategies often interact (Cardina et al., 2002). As such, the cropping system (which includes both the crops grown in rotation and the associated cultural practices) is the best reference term to frame risk mitigation measures for the use of GMHT crops.

Alternatively, the efficacy in use of herbicides can be optimised within a cropping system (Wilson et al., 2011). This can be achieved by scouting for weeds, integrating knowledge of weed biology and ecology, using appropriate application technologies, and by applying suitable herbicide regimes (Nazarko et al., 2005; Parker et al., 2006). In this respect, new or existing herbicidal active substances may be targeted to fill the gaps in the activity spectrum of glyphosate. There is also the possibility to redesign cropping systems in a manner that reduces the size and interference capacity of weeds (Nazarko et al., 2005). The EFSA GMO Panel acknowledges that the transition to integrated weed management will depend upon a wide range of technical, economic and socio-economic factors (Meissle et al., 2010; Vasileiadis et al., 2011). A clear advantage of focussing on increased cropping system diversification is that it would increase or conserve farmland biodiversity, and reduce the risk of weed shifts and of evolution of glyphosate resistant weed biotypes.

The EFSA GMO Panel pinpoints the importance of providing farmers with sufficient information so that they understand the reasons for adopting integrated weed management programmes and the need to utilise best management practices, especially in those situations where weed resistance is most likely to evolve (Table 2). It is also advisable that weed resistance management is considered for the implementation of integrated pest management principles, as foreseen under Directive 2009/128/EC. Product stewardship programmes, technical guides and label recommendations as proposed by the applicant can help educate farmers to effectively manage the evolution of glyphosate resistant weeds and to develop sustainable long-term management strategies (Sammons et al., 2007; Owen et al., 2011; Shaner et al., 2011).

In its evaluation, the CZ CA was of the opinion that "*measures should be put in place under Directive 91/414/EEC and consecutively under Regulation (EC) No 1107/2009 to ensure compliance with regulatory requirements for the pesticide regimes used in Member States. These should include measures for the development of weed resistance management strategies in each Member State permitting the use of glyphosate on maize GA21. It is necessary to exploit properly the appropriate antiresistance strategy to avoid undue herbicide usage*" (section 7.6.2 of the environmental risk assessment report of the CZ CA). The CZ CA recommended the applicant "*to design appropriately a relevant Technical User Guide for farmers that should involve Good Agricultural Practices of glyphosate applications guaranteeing sustainable and safe use of the entire GA21 technology*" (section 9 of the environmental risk assessment report of the CZ CA).

6.3.1.4. Conclusion on risk mitigation measures

The EFSA GMO Panel considered several risk mitigation measures that can be put in place to reduce the risks that the cultivation of maize GA21 may pose to the environment (section 6.3.1.3 and Table 2). In practice, it is the responsibility of risk managers to decide upon risk mitigation measures that are consistent with the environmental protection goals and biodiversity action plans pertaining to their regions, and that are proportionate to the levels of risk and scientific uncertainty identified in the environmental risk assessment.

The EFSA GMO Panel anticipates that the repeated use of glyphosate at recommended application rates on continuous maize GA21 and/or other glyphosate tolerant crops grown in rotation may result in reductions in botanical diversity and/or weed density in maize fields to a level that might adversely affect food chains and webs, but not necessarily biological control functions, at the field and landscape level. Such a reduction in biodiversity may be considered problematic by risk managers depending upon protection goals pertaining to their region, especially in receiving environments that sustain little farmland biodiversity or in environmentally sensitive areas. Under such situations, the EFSA GMO Panel recommends that risk mitigation measures are put in place to manage potential herbicide effects, in order to ensure that glyphosate on maize GA21 is used within diversified cropping regimes that have similar or reduced adverse effects on farmland biodiversity compared with conventional maize cultivation. Possible risk mitigation measures include protecting adjacent habitats from herbicide drift, (re)introduction and better management of field margins or other ‘out of crop’ measures, less intense in-crop weed management, and especially rotating crops.

The cultivation of maize GA21 in monoculture or in rotation with other glyphosate tolerant crops, in conjunction with the repeated and/or exclusive application of glyphosate-based herbicides will cause changes in weed flora, and will favour the evolution and spread of glyphosate resistant weeds due to the selection pressure exerted by glyphosate. This, in turn, may affect food webs, and the functional value of weed vegetation for organisms of higher trophic levels (reduced functional biodiversity). The selection pressure on weeds can be reduced by crop rotations (i.e., rotating glyphosate tolerant crops with non-glyphosate tolerant crops), using variable application rates and timing, applying a variety of herbicidal active substances with different modes of action, and by using non-herbicide weed control tools such as post-emergence cultivation and cover crops. To be most effective, these methods should be used in combination. A clear advantage of increasing cropping system diversification is that it would increase or conserve farmland biodiversity, as well as reducing the risk of weed shifts and the evolution of glyphosate resistant weed biotypes.

In its evaluation, the CZ CA was of the opinion that “*measures should be put in place under Directive 91/414/EEC and consecutively under Regulation (EC) No 1107/2009 to ensure compliance with regulatory requirements for the pesticide regimes used in Member States. These should include measures for the appropriate management of glyphosate on GMHT maize and for the development of weed resistance management strategies in each Member State permitting the use of glyphosate on maize GA21. It is necessary to exploit properly the appropriate antiresistance strategy to avoid undue herbicide usage*” (section 7.6.2 of the environmental risk assessment report of the CZ CA). The CZ CA recommended the applicant “*to design appropriately a relevant Technical User Guide for farmers that should involve Good Agricultural Practices of glyphosate applications guaranteeing sustainable and safe use of the entire GA21 technology*” (section 9 of the environmental risk assessment report of the CZ CA).

6.3.2. Post-market environmental monitoring[63]

6.3.2.1. General aspects of post-market environmental monitoring

Directive 2001/18/EC introduces an obligation for applicants to implement monitoring plans, in order to trace and identify any direct or indirect, immediate, delayed or unanticipated effects on human health or the environment of GMOs as or in products after they have been placed on the market. Monitoring plans should be designed according to Annex VII of the Directive. According to Annex VII, the objectives of an environmental monitoring plan 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.

Post-market environmental monitoring is composed of case-specific monitoring and general surveillance (EFSA, 2006b, 2011c). Case-specific monitoring is not obligatory, but may be required to verify assumptions and conclusions of the environmental risk assessment, whereas general surveillance is mandatory, in order to take account of general or unspecified scientific uncertainty and

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[63] Technical dossier / Section D11 / Appendices 29 and 30 // Additional information received on 13 July 2009 / Appendix 9

any unanticipated adverse effects associated with the release and management of a GM plant. Due to different objectives between case-specific monitoring and general surveillance, their underlying concepts differ (Sanvido et al., 2005). Case-specific monitoring should enable the determination of whether and to what extent adverse effects anticipated in the environmental risk assessment occur during the commercial use of a GM plant, and thus to relate observed changes to specific risks. It is triggered by scientific uncertainty that was identified in the environmental risk assessment. As a consequence, a hypothesis can be established that can be tested on the basis of newly-collected monitoring data.

The objective of general surveillance is to identify unanticipated adverse effects of the GM plant or its use on human health and the environment that were not predicted or specifically identified during the environmental risk assessment. In contrast to case-specific monitoring, the general status of the environment that is associated with the use of the GM plant is monitored without any preconceived hypothesis, in order to detect any possible effects that were not anticipated in the environmental risk assessment, or that are long-term and cumulative. Should any such effects be observed, they are studied in more detail to determine whether the effect is adverse and whether it is associated with the use of the GM plant (Sanvido et al., 2005, 2009, 2011a,b; EFSA, 2006b, 2011c).

6.3.2.2. Interplay between environmental risk assessment, mitigation and post-market environmental monitoring

With the consideration of risk mitigation measures, the environmental risk assessment of maize GA21 concluded that the cultivation of maize GA21 may result in adverse environmental effects due to the use of the complementary glyphosate-based herbicides. These potential adverse environmental effects comprise (1) a reduction in farmland biodiversity, (2) changes in botanical diversity due to weed shifts, with the selection of weed communities mostly composed of tolerant species, and (3) the selection of glyphosate resistant weeds. The magnitude of these potential adverse environmental effects depends upon a range of environmental and management factors and the EFSA GMO Panel proposed several risk mitigation measures to reduce environmental impacts to those of comparable conventional maize cultivation or to meet the protection goals of different farming regions. However, the practicality and implementation of these measures will vary according to local conditions and so there is scientific uncertainty as to whether they will achieve the desired goals.

6.3.2.3. Case-specific monitoring[64]

When potential adverse effects or important gaps in scientific information or significant levels of critical uncertainty linked to the GM plant and its management have been identified in the environmental risk assessment, then case-specific monitoring should be carried out after placing on the market, in order to confirm assumptions made in the environmental risk assessment and to further inform the environmental risk assessment. Case-specific monitoring should be targeted at assessment endpoints and environmental protection goals identified as being at risk during the environmental risk assessment, or where levels of critical uncertainty were identified in relation to potential risks associated with the GM plant (EFSA, 2011c).

The specific risks identified in section 6.2.8 (conclusion on the environmental risk assessment) are (1) a reduction in farmland biodiversity due to novel herbicide regimes, (2) changes in botanical diversity due to weed shifts, with the selection of weed communities mostly composed of tolerant species due to novel herbicide regimes, and (3) the selection of glyphosate resistant weeds due to novel herbicide regimes. The EFSA GMO Panel notes that for these risks the possible environmental effects are related to the use of the complementary herbicide, and judges that monitoring could equally well be put in place either under the legislation for plant protection products (Regulation (EC) No 1107/2009, which replaced Directive 91/414/EEC on 14 June 2011, and Directive 2009/128/EC), or under the legislation for GMOs (Directive 2001/18/EC). In reaching this view, the EFSA GMO Panel considered: the interplay between the legislation for GMOs and plant protection products

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(section 6.2.7.1); the fact that some herbicide tolerant systems on the market are non-GM; and the fact that protection goals are set at Member State level. However, since the remit of the EFSA GMO Panel to propose monitoring is linked inextricably to Directive 2001/18/EC, subsequent recommendations in this section are based on GMO legislation; the terminology 'case-specific monitoring' is therefore used in that context.

In considering the form that case-specific monitoring should take, the EFSA GMO Panel reiterates the considerable challenges it identified previously (EFSA, 2009c, 2011e) to the drawing of meaningful conclusions on the environmental consequences of the use of herbicides from large-scale multi-site experiments, such as the FSEs, which seek to compare HT with conventional herbicide management (Squire et al., 2003, 2009). On the grounds of scientific practicability (e.g., Perry et al., 2003) and of cost (e.g., Qi et al., 2008), and the fact that glyphosate is already extensively used in a wide range of crops, such studies are considered disproportionate to the identified risks.

In order to assess the efficacy of risk mitigation measures put in place to reduce levels of risk and in order to reduce the remaining scientific uncertainty, the EFSA GMO Panel recommends case-specific monitoring to address (1) changes in botanical diversity within fields due to novel herbicide regimes, and (2) resistance evolution to glyphosate in weeds due to novel herbicide regimes.

Monitoring changes in botanical diversity within fields due to novel herbicide regimes

The EFSA GMO Panel recommends that the applicant proposes a detailed stewardship scheme to farmers for the use of glyphosate on maize GA21. This scheme should recommend detailed herbicide/cropping regimes that are environmentally sustainable and no more harmful to botanical diversity than the current conventional management practices within each receiving environment, according to local environmental protection goals. The applicant should provide explicit justification based on data from field trials/experiments and from on-farm demonstrations concerning the efficacy of these regimes compared to the baseline, for each receiving environment. At early stages of commercialisation, the justification for the safety of the applicant's proposed regimes may be supported by field trials and farmer demonstrations that usually accompany the introduction of new agrochemicals and new technology into agriculture. Local experimental or demonstration sites are already in place in several Member States to assess the impact of various crop protection programmes, including integrated pest management strategies (Fried et al., 2009; Cordeau et al., 2011), and could also be used to assess the impact of glyphosate-based regimes on the level of botanical odiversity.

The EFSA GMO Panel recommends that monitoring be put in place to assess that the proposed herbicide/cropping regimes recommended by the applicant are implemented satisfactorily for maize GA21 and that they have the proposed efficacy to ensure that any adverse effects on biodiversity are no greater than those caused by conventional management. This may be achieved during the cultivation of maize GA21 through a combination of collection of additional information from the farmer questionnaires (section 6.3.2.4) on herbicide and crop management practices and on weed populations, and from a strictly limited number of more specific and focussed multi-annual scientific studies at sites where adequate baselines have already been established. In addition, case-specific monitoring is recommended to monitor the efficacy of any of those measures discussed generally in section 6.3.1 and specified in section 6.3.1.4 above adopted to mitigate harm to biodiversity.

The responsibility for the generation of a monitoring methodology for determining the efficacy of such regimes rests properly with the applicant. However, the EFSA GMO Panel recommends that (1) whatever monitoring methodology is chosen, it is likely that it would benefit from a close collaboration between the applicant and the research community. Scientists with relevant expertise in this area (e.g., ecologists, weed scientists) should be consulted, (2) variation amongst local protection goals implies that Member State involvement in planning is essential, (3) adequate baselines be established prior to the introduction of the GMHT cropping systems, to enable changes to be detected, (4) both grain and forage maize should be considered, if appropriate, (5) conclusions should be drawn not only for a single season but also at the temporal scale of a complete rotation, (6) measurement endpoints should be selected to confirm the preservation of functional biodiversity sufficient to guarantee the quality of agro-ecosystems systems and ensure their sustainability (Storkey et al., 2008; EFSA, 2010d,e).

The conclusion of the EFSA GMO Panel is consistent with the evaluation carried out by the CZ CA on maize GA21. The CZ CA considered that "*case-specific monitoring is the most appropriate way to monitor identified potential environmental effects that could result from changes in the technology. Therefore it is proposed that the applicant performs a case-specific monitoring of long-term effects with focus on non-target organisms and microbial biodiversity*" (section 8.1 of the environmental risk assessment report of the CZ CA).

Monitoring resistance evolution to glyphosate in weeds due to novel herbicide regimes

Since glyphosate is a widely used herbicide and managing resistance evolution is a condition of its registration as a pesticide (section 6.2.7.3), the EFSA GMO Panel advises that the use of glyphosate on GMHT crops, including maize GA21, is integrated in the monitoring conducted by the applicant in relation to all uses of glyphosate within Member States.

The EFSA GMO Panel recommends that applicants establish stewardship systems which encourage farmers to report weed control failures to them as required under Regulation (EC) No 1107/2009. Applicants will need to liaise with other providers of glyphosate-based herbicides and also with the producers of other glyphosate tolerant crops. Such observations may reveal the occurrence of localised resistance before it spreads, and may serve as a trigger for further investigations (Shaner, 2010).

In addition, risk managers should consider additional routine monitoring for weed resistance in areas where the risk of resistance evolution is highest, i.e., in areas of continuous or repeated use of glyphosate (not solely on maize GA21) as described in section 6.2.7.3.

The EFSA GMO Panel considers that farmer questionnaires provide an opportunity for farmers to report weed control failures or declines in the efficacy of glyphosate. In addition, farmers will indicate their herbicide regimes, and so it will be possible to determine whether they are implementing resistance management strategies and following stewardship guidelines (section 6.3.2.4).

Weed resistance should be reported to each Member State on an annual basis, and these national reports can then be submitted to organisations such as the European Weed Research Society (EWRS), that has Working Groups monitoring weed resistance and developing integrated weed management strategies aimed also to delay or manage weed resistance to herbicides.

The conclusion of the EFSA GMO Panel is consistent with the evaluation carried out by the CZ CA on maize GA21. The CZ CA considered that "*case-specific monitoring is the most appropriate way to monitor identified potential environmental effects that could result from changes in the technology. Therefore it is proposed that the applicant performs a case-specific monitoring of long-term effects with focus on weed shifts and the development of herbicide resistance to glyphosate*" (section 8.1 of the environmental risk assessment report of the CZ CA).

6.3.2.4. General surveillance[65]

According to Directive 2001/18/EC, the objective of general surveillance is to detect any unanticipated adverse effects on protected and valued entities of the environment that may be due to the cultivation of GM plants, including biodiversity and ecosystem services (EFSA, 2011c).

The applicant proposed to conduct general surveillance for maize GA21 throughout the period of validity of the authorisation. The general surveillance will take into consideration and be proportionate to the extent of cultivation of maize GA21 in the EU Member States. The applicant proposed to build its general surveillance on four approaches (1) the use of annual farmer questionnaires, (2) the review

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of scientific information provided by existing monitoring networks, (3) the monitoring and review of ongoing research and development, as well as scientific literature, and (4) the implementation of industry stewardship programs, in order to identify potential adverse effects associated with the intended uses of maize GA21.

Farmer questionnaires[66]

The EFSA GMO Panel agrees with the general surveillance approach of the applicant to establish farmer questionnaires as a reporting format that provides relevant information. The questionnaires to farmers exposed to or using GM plants are regarded by the EFSA GMO Panel as an adequate tool for addressing several aspects of general surveillance (EFSA, 2006b, 2011c). The EFSA GMO Panel is of the opinion that farmer questionnaires enable the reporting of any on-farm observations of effects associated with the cultivation of maize GA21, as this approach uses first-hand observations and rely on farmers' knowledge and experience of their local agricultural environments, comparative crop performance and other factors that may influence events on their land (Schmidt et al., 2008; Wilhelm et al., 2010). Some of the questions link directly to assessment endpoints or give indirect indications of effects on assessment endpoints (EFSA, 2011c).

Farmer questionnaires should be designed to determine whether the farmer/manager/worker has noticed any differences between the GM plant and its management and that of similar non-GM plants growing on the farm, nearby or previously (EFSA, 2011c). The applicant and risk managers are advised to consider the new EFSA GMO Panel guidelines on post-market environmental monitoring (EFSA, 2011c) and the specific recommendations on the annual post-market environmental monitoring report of maize MON 810 cultivation in 2009 (EFSA, 2011d) when finalising their or evaluating monitoring plans.

While the EFSA GMO Panel considers the format and contents of the farmer questionnaire, as provided by the applicant, comprehensive, it proposes the following modifications:

- to add questions on the possible occurrence and observation of (GM) volunteer maize in subsequent crops and feral maize plants (if any) in field margins for the consideration of unanticipated effects on the persistence and invasiveness potential of maize GA21;
- in addition to the relative impact on main weeds, the farmer questionnaire should specifically request information on the active substances (or commercial product names), dosage and timing of herbicide applications, as well as on the use of non-chemical direct weed control methods, as a range of weed control strategies could be adopted with different environmental impacts. Moreover, it will deliver relevant information that allows checking adoption of national/regional measures to mitigate the potential reduction of in-field botanical diversity due to use of glyphosate on maize GA21. If herbicides containing glyphosate are used in that field at any point in the crop rotation, either in-crop or inter-crop, this should also be recorded (Castellazzi et al., 2007, 2008);
- to consider unexpected effects on beneficial ecological functions provided by soil microbial communities due to the specific use of glyphosate-based herbicides during the growing season of maize GA21 or other glyphosate tolerant plants (see Smit et al., 2012 for indirect indicators in terms of yield, fertiliser use, quality).

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[66] Additional information received on 27 October 2009 / Question 4 / Pages 10-12 / Appendix 2

In line with the general recommendations on the farmer questionnaire set in its 2011 Scientific Opinion on post-market environmental monitoring (EFSA, 2011c), the EFSA GMO Panel advises farmer questionnaires:

- are designed to ensure the appropriate statistical validity and representativeness of the collected data, including the proportion of fields growing maize GA21 in a region and a minimum percentage or number of questionnaires required to achieve statistical power in the data collected;
- are designed to generate data on the agronomic management of maize GA21, as well as data on impacts on farming systems and the farm environment;
- use a field or group of fields growing maize GA21 as the basic unit for monitoring in representative farming regions and for representative cropping systems within the country. The precise fields should be identified, so that their locations can be subsequently retrieved from registers of GM plant sites;
- clearly identify the comparator (e.g., variety, location) and whether it is being grown adjacent to maize GA21, on the same farm or in another location. If no comparators are being grown spatially or temporally close to maize GA21, then the rationale for selecting another comparator (e.g., historical data) should be fully described;
- where appropriate, observe the field/fields in subsequent years for any unusual residual effects;
- provide information on other GM plant events being grown at the same sites and farms;
- are adapted, where needed, to each GM plant monitoring on a case-by-case basis by considering additional data requirements relevant for each species/event, its management and its receiving environments;
- are user friendly but also information rich;
- are constructed to encourage independent and objective responses from farmers, land managers and others involved with maize GA21 or its transgene products;
- are audited to ensure the independence and integrity of all monitoring data.

In addition to the general recommendations on the farmer questionnaire (EFSA, 2011c) and in line with its 2011 Scientific Opinion on the annual post-market environmental monitoring report on maize MON 810 cultivation in 2009 (EFSA, 2011d), the EFSA GMO Panel advises the applicant to take into account the following points:

- the sampling frame should be comprehensive and a stratification should be applied consistently in each country. Adequate sampling should be carried out from the previous stratification exercise;
- the cultivation areas, with high uptake of maize GA21 and where maize GA21 has been continuously grown in previous years, should be over-represented in the sampling scheme;
- the number of farmers not participating in the survey and the reasons thereof should be documented;
- impartial and standardised interviews should be carried out by independent parties and effective quality and auditing procedures should be considered;
- additional questions to the farmer questionnaire should be considered to better describe the cultivation of maize in the local area and/or the previous years, the receiving environments and the management systems in which maize GA21 is being grown;

- relevant data as from other sources of information (e.g., official statistics on crop management practices) should/could be considered for validity check of the questionnaires (e.g., consistency, representativeness);

- the raw data, programmes, logs and output files related to the statistical analysis of the farmer questionnaires should be provided. Confidence intervals for the analysis of the monitoring characteristics should be included in the statistical report;

- appropriate statistical procedures should be used based on using a distribution for appropriate outcomes;

- the use of a standard default effect size of 5 % is not relevant for all assessment endpoints and, where scientifically justified, different default effect sizes should be considered for some assessment endpoints;

- data should be pooled and statistically analysed over years. At the end of the ten years of general surveillance, the applicant should conduct a statistical analysis with all pooled data;

- a codification for farmers repeatedly surveyed over years should be set up. These farmers should be particularly monitored;

- the number of years the surveyed farmer has grown maize GA21 and other GM plants should be indicated.

The CZ CA considered that "*the general surveillance plan should be updated*" and made specific proposals to strengthen the farmer questionnaire (section 8.2 of the environmental risk assessment report of the CZ CA).

Existing monitoring networks

Since farmer questionnaires focus mainly on the cultivation area of the GM plant and its surroundings, the EFSA GMO Panel supports the consideration of additional information sources for general surveillance (EFSA, 2006b, 2011c). In this respect, Directive 2001/18/EC proposed to make use of established routine surveillance networks, in order to obtain data on environmental impacts in the landscape where GMOs are cultivated from a range of existing monitoring networks which observe changes in biota and production practices from farm up to regional level. EU Member States have various networks in place – some of which have a long history of data collection – that may be helpful in the context of general surveillance of GM plant cultivations. Existing monitoring networks involved in routine surveillance offer recognised expertise in a specific domain and have the tools to capture information on important environmental aspects over a large geographical area. However, the EFSA GMO Panel recognises that existing monitoring networks fully meeting all the needs of the monitoring of GM plant cultivations can be limited (Bühler, 2006; Mönkemeyer et al., 2006; Schmidtke and Schmidt, 2007; Graef et al., 2008; Smit et al., 2012). The development of harmonised criteria for the systematic identification, specification and analysis of existing surveillance networks across the EU is therefore considered important (EFSA, 2011c).

The EFSA GMO Panel agrees with the proposal of the applicant to describe the generic approaches for using existing monitoring networks. The applicant has also given consideration to the use of any future surveys of conservation goals as defined in the Directive 2004/35/EC on environmental liability (EC, 2004) in farming regions where maize GA21 will be cultivated and intends to investigate their suitability for providing data on potential changes in biota.

Knowing the limitations of existing monitoring networks, it is important to describe the processes and criteria that will be used for selecting and evaluating existing monitoring networks for supplying data related to the unanticipated adverse effects of GM plants in general surveillance. Therefore, the applicant, in consultation with Member States, should:

- consider the protection goals, the assessment endpoints and their indicators that could be monitored through existing monitoring programmes;
- identify the type of existing monitoring networks that would be appropriate to survey the protection goals considered to be at risk in the countries where maize GA21 will be grown;
- describe the generic approach and develop more detailed criteria to evaluate existing monitoring networks and how appropriate networks will be selected (considering the hereunder list of points);
- identify what changes need to be made to these monitoring networks and describe how these might be implemented, and identify gaps in information that could be filled by additional surveys;
- encourage these networks to adopt the proposed modifications and describe how data from these networks will be integrated and assessed.

In addition, when selecting existing monitoring networks to be part of general surveillance, the applicant is recommended to consider the following points for assessing the suitability of these existing networks to supply relevant general surveillance data:

- the relevance of protection goals and their indicators monitored through existing monitoring networks;
- the type (e.g., raw data) and quality of the data recorded;
- the statistical power and the effect sizes detected by monitoring networks, where appropriate;
- the ease of access to the data collected by existing monitoring networks (e.g., availability of data via Internet, free access to data or access subject to a fee, protected data of ongoing research projects);
- the track record and past performance of existing monitoring networks;
- the methodology used by existing monitoring networks (e.g., sampling and statistical approach) including (1) the spatial scale of data collection (e.g., local, regional, national, zonal): existing monitoring networks focusing on agricultural areas cultivated with GM plants or with conventional plants like maize, potato (for which GM are also available and grown) should be preferred, (2) temporal scale of data collection: appropriate frequency of data collection and reporting (e.g., short-term vs. long-term data sets, regularity of data collection), and (3) other parameters such as the language of the reports, impartiality.

Furthermore, the EFSA GMO Panel recommends that the applicant describes arrangements with any third parties participating in its general surveillance plan. It is recommended to consider how arrangements for collecting, collating and analysing data will be made, and to describe how formal agreements, procedures and communication will be established with the European Commission and Member States or other third parties, although detailed arrangements may not have been agreed at the time of the application.

The EFSA GMO Panel also recommends to include in the sources of information that support general surveillance of maize GA21, existing monitoring networks that monitor herbicide usage, botanical diversity on farms and weed resistance evolution, so that the scientific requirements for the detection of any unforeseen environmental effects due to altered farm management practices associated with maize GA21 cultivation are met.

The applicant provided a separate general surveillance plan for the import and processing of maize GA21[67]. This plan was previously evaluated by the EFSA GMO Panel (EFSA, 2007), and includes (1) the description of an approach involving operators (federations involved in maize import and processing), reporting to applicants, via a centralised system, any observed adverse effect(s) of GMOs on human health and the environment, (2) a coordinating system established by EuropaBio for the collection of the information recorded by the various operators, and (3) the use of networks of existing monitoring networks (Lecoq et al., 2007; Windels et al., 2008).

The CZ CA considered that "*the general surveillance plan should be updated*" and made specific proposals to strengthen the involvement of monitoring networks (section 8.2 of the environmental risk assessment report of the CZ CA).

Monitoring and review of ongoing research and development, as well as scientific literature

An additional approach to support general surveillance is to review all new scientific, technical and other information pertaining to maize GA21, including information on GM plants with similar traits or characteristics, which has emerged during the reporting period. This will include reviewing of results from ongoing research and development studies (e.g., variety registration trials) and all publications including peer-reviewed journal articles, conference proceedings, review papers and any additional studies or other sources of information relevant to the cultivation of the plant/trait combination for which the report is being drafted (EFSA, 2011c).

The EFSA GMO Panel recommends the applicant:

- to cover all relevant peer-reviewed publications, including peer-reviewed journal articles, conference proceedings, review papers and any additional studies or other sources of information relevant to the cultivation of the plant/trait combination for which the report is being drafted;
- to describe the criteria for selecting and evaluating the scientific reliability of publications;
- to adhere to systematic literature review methodology to select relevant papers (EFSA, 2010f).

Industry stewardship programs

The EFSA GMO Panel welcomes the applicant's proposal to develop stewardship programs for the introduction, marketing, management and stewardship of maize GA21, but advises that these programmes should be made available well in advance of the time of commercialisation so as to allow risk managers to validate the implementation of proportional risk management measures and detailed monitoring plans.

6.3.2.5. Reporting results of post-market environmental monitoring[68]

The applicant will submit a report on an annual basis covering case-specific monitoring and general surveillance. In case of adverse effects altering the conclusions of the environmental risk assessment, the applicant will immediately inform the European Commission and Member States. The EFSA GMO Panel agrees with the proposal made by the applicant on reporting intervals. The EFSA GMO Panel recommends that effective reporting procedures are established with the Competent Authorities of Member States and the European Commission as required under the Council Decision 2002/811/EC on monitoring.

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[67] Technical dossier / Section D11.4 / Appendix 29

[68] Technical dossier / Section D11.5

The results of post-market environmental monitoring should be presented in accordance with the standard reporting formats established by the 2009/770/EC Commission Decision on standard reporting formats. In addition, the applicant is recommended to provide raw data, in order to allow different analyses and interrogation of the data and to allow scientific exchange and co-operation between Member States, the European Commission and EFSA. The EFSA GMO Panel recommends that the applicant describes whether the post-market environmental monitoring reports contain cumulative analyses of data with previous years' results.

6.3.2.6. Conclusion on post-market environmental monitoring

The EFSA GMO Panel gave its opinion and made recommendations on the scientific quality of the post-market environmental monitoring plan proposed by the applicant. In order to assess the efficacy of risk mitigation measures put in place to reduce levels of risk and in order to reduce the remaining scientific uncertainty identified in the environmental risk assessment, the EFSA GMO Panel recommends case-specific monitoring to address (1) changes in botanical diversity within fields due to novel herbicide regimes, and (2) resistance evolution to glyphosate in weeds due to novel herbicide regimes. The EFSA GMO Panel considers that risk managers should adapt monitoring methodologies to their local receiving environments and management systems.

The EFSA GMO Panel agrees with the general surveillance plan for the cultivation of maize GA21 of the applicant (1) to establish farmer questionnaires as a reporting format of any on-farm observations of effects associated with the cultivation of maize GA21, (2) to use existing monitoring networks which observe changes in biota and production practices from farm up to regional level to obtain data on environmental impacts in the landscape where maize GA21 is cultivated, (3) to review all new scientific, technical and other information pertaining to maize GA21, and (4) to develop stewardship programs for the introduction, marketing, management and stewardship of maize GA21, but requests that its proposals and those made by the CZ CA to strengthen general surveillance are implemented. The EFSA GMO Panel agrees with the reporting intervals and modalities proposed by the applicant. The general surveillance plan for the import and processing of maize GA21 was previously evaluated by the EFSA GMO Panel (EFSA, 2007).

The CZ CA considered that "*case-specific monitoring is the most appropriate way to monitor identified potential environmental effects that could result from changes in the technology. Therefore it is proposed that the applicant performs a case-specific monitoring of long-term effects with focus on non-target organisms, weed shifts, the development of herbicide resistance to glyphosate and microbial biodiversity*" (section 8.1 of the environmental risk assessment report of the CZ CA).

The CZ CA considered that "*the general surveillance plan should be updated*" and made specific proposals to strengthen general surveillance (section 8.2 of the environmental risk assessment report of the CZ CA).

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Following the submission of an application (Reference EFSA-GMO-UK-2008-60) 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 herbicide tolerant genetically modified (GM) maize GA21 (Unique Identifier MON-ØØØ21-9) for food and feed uses, import, processing and cultivation.

In delivering its Scientific Opinion, the EFSA GMO Panel considered the application EFSA-GMO-UK-2008-60, additional information supplied by the applicant, scientific comments submitted by Member States, the environmental risk assessment report of the Czech Competent Authority (CZ CA), and relevant scientific publications.

Maize GA21 expresses a modified version of 5-enolpyruvylshikimate-3-phosphate synthase (mEPSPS), which is derived from maize EPSPS, and renders maize GA21 tolerant to the herbicidal active substance glyphosate.

The EFSA GMO Panel evaluated maize GA21 with reference to its intended uses and appropriate principles described in its guidelines for the risk assessment of GM plants and derived food and feed, the environmental risk assessment of GM plants, the selection of comparators for the risk assessment of GM plants, and for the post-market environmental monitoring of GM plants. The scientific evaluation of the risk assessment included molecular characterisation of the inserted DNA and expression of target protein. An evaluation of the comparative analyses of composition, agronomic and phenotypic characteristics was undertaken, and the safety of the new protein, and the whole food/feed was evaluated with respect to potential toxicity, allergenicity and nutritional quality. An evaluation of environmental impacts and the post-market environmental monitoring plan was undertaken.

The molecular characterisation data establish that maize GA21 contains a single insertion locus consisting of six contiguous complete or truncated versions of the m*epsps* expression cassette used for the transformation. No other parts of the plasmid are present in the transformed plant. Bioinformatic analyses of the open reading frames spanning the junction sites within the insert or between the insert and genomic DNA did not indicate specific hazards. The stability of the inserted DNA and the herbicide tolerance trait were confirmed over several generations. Updated analyses of the levels of mEPSPS in various plant parts collected from field trials performed in Europe were considered sufficient.

Based on the results of comparative analysis, the EFSA GMO Panel concludes that maize GA21 is compositionally, phenotypically and agronomically not different from the conventional counterpart, except for the presence of the mEPSPS protein in maize GA21. Bioinformatics studies demonstrated that the newly expressed mEPSPS protein shows no homology to known toxic proteins or allergens. The *in vitro* digestibility studies showed that most of the protein was degraded. There were no indications of adverse effects after administration of grain from maize GA21 to rats in a repeated-dose 90-day oral toxicity study. A feeding study with broiler chickens confirmed that grain from maize GA21 is as nutritious as grain of the conventional counterpart and a reference maize variety. Based on the available information, the EFSA GMO Panel is of the opinion that maize GA21 is as safe and nutritious as the conventional counterpart and reference maize varieties, and that it is unlikely that the overall allergenicity of the whole plant is changed.

Since the scope of the current application covers cultivation, the environmental risk assessment considered the environmental impact of full-scale commercialisation of maize GA21.

The CZ CA (including its Biosafety Commission) provided to EFSA its report on the environmental risk assessment of maize GA21 (dated 20 October 2010) on 25 October 2010 in line with Articles 6.3(c) and 18.3(c) of Regulation (EC) No 1829/2003. The report on the environmental risk assessment of the CZ CA is provided in Annex H of the EFSA Overall Opinion, and has been considered throughout this EFSA GMO Panel Scientific Opinion.

The EFSA GMO Panel considers that maize GA21 has no altered agronomic and phenotypic characteristics, except for the herbicide tolerance. The likelihood of unintended environmental effects due to the establishment, survival and spread of maize GA21 is considered to be extremely low, and will be no different from that of conventional maize varieties.

It is highly unlikely that the recombinant DNA will transfer and establish in the genome of bacteria in the environment or human and animal digestive tracts. In the rare but theoretically possible case of transfer of the m*epsps* gene from maize GA21 to soil bacteria, no novel property would be introduced into the soil bacterial community and thus no positive selective advantage that would not have been conferred by natural gene transfer between bacteria would be provided.

Based on the evidence provided by the applicant and relevant scientific literature on maize GA21, the EFSA GMO Panel concludes that there are no indications of adverse effects on non-target organisms due to unintended changes in maize GA21, and therefore considers *trait*-specific information appropriate to assess whether maize GA21 poses a risk to non-target organisms.

The studies, supplied or reviewed by the applicant, showed no adverse effects on different types of non-target organisms due to the expression of the mEPSPS protein in glyphosate tolerant crops.

The EFSA GMO Panel does not expect potential adverse effects on biogeochemical processes and the abiotic environment due to the expression of the mEPSPS protein in maize GA21.

The EFSA GMO Panel is of the opinion that potential adverse environmental effects of the cultivation of maize GA21 are associated with the use of the complementary glyphosate-based herbicide regimes. These potential adverse environmental effects comprise (1) a reduction in farmland biodiversity, (2) changes in botanical diversity due to weed shifts, with the selection of weed communities mostly composed of tolerant species, and (3) the selection of glyphosate resistant weeds. The potential harmful effects could occur at the level of arable weeds, farmland biodiversity, food webs and the ecological functions they provide. The magnitude of these potential adverse environmental effects will depend upon a series of factors, including the specific herbicide and cultivation management applied at the farm level, the crop rotation and the characteristics of receiving environments.

The EFSA GMO Panel considers that the use of glyphosate-based herbicides at recommended field application rates of glyphosate on maize GA21 is unlikely to cause adverse effects to soil microbial communities or beneficial functions mediated by them.

The conclusions of the EFSA GMO Panel on the environmental safety of maize GA21 are consistent with those of the CZ CA. The CZ CA concluded that "*based on the existing information and data provided by the Syngenta Company within the evaluation process, the Czech CA considers that maize GA21 has no altered survival, multiplication or dissemination characteristics and interacts with other organisms as any conventional maize. However, the data presented on the issue of "Impacts of the specific cultivation, management and harvesting techniques" do not allow a comprehensive assessment of potential long-term effects on the environment associated to the use of the herbicide*" (section 9 of the environmental risk assessment report of the CZ CA). Hence, the CZ CA identified "*no potential effects on the environment either immediate, delayed, direct or indirect with the exception of those related to the change in the herbicide management*" (section 8.1 of the environmental risk assessment report of the CZ CA).

The EFSA GMO Panel anticipates that the repeated use of glyphosate at recommended application rates on continuous maize GA21 and/or other glyphosate tolerant crops grown in rotation may result in reductions in botanical diversity and/or weed density in maize fields to a level that might adversely affect food chains and webs, but not necessarily biological control functions, at the field and landscape level. Such a reduction in biodiversity may be considered problematic by risk managers depending upon protection goals pertaining to their region, especially in receiving environments that sustain little farmland biodiversity or in environmentally sensitive areas. Under such situations, the EFSA GMO Panel recommends that risk mitigation measures are put in place to manage potential herbicide effects, in order to ensure that glyphosate on maize GA21 is used within diversified cropping regimes that have similar or reduced adverse effects on farmland biodiversity compared with conventional maize cultivation. Possible risk mitigation measures include protecting adjacent habitats from herbicide drift, (re)introduction and better management of field margins or other 'out of crop' measures, less intense in-crop weed management, and especially rotating crops.

The cultivation of maize GA21 in monoculture or in rotation with other glyphosate tolerant crops, in conjunction with the repeated and/or exclusive application of glyphosate-based herbicides will cause changes in weed flora, and will favour the evolution and spread of glyphosate resistant weeds due to the selection pressure exerted by glyphosate. This, in turn, may affect food webs, and the functional value of weed vegetation for organisms of higher trophic levels (reduced functional biodiversity).

Under such situations, the EFSA GMO Panel recommends that risk mitigation measures are put in place to delay resistance evolution. The selection pressure on weeds can be reduced by crop rotations (i.e., rotating glyphosate tolerant crops with non-glyphosate tolerant crops), using variable application rates and timing, applying a variety of herbicidal active substances with different modes of action, and by using non-herbicide weed control tools such as post-emergence cultivation and cover crops. To be most effective, these methods should be used in combination. A clear advantage of increasing cropping system diversification is that it would increase or conserve farmland biodiversity, as well as reducing the risk of weed shifts and the evolution of glyphosate resistant weed biotypes.

In its evaluation, the CZ CA was of the opinion that "*measures should be put in place under Directive 91/414/EEC and consecutively under Regulation (EC) No 1107/2009 to ensure compliance with regulatory requirements for the pesticide regimes used in Member States. These should include measures for the appropriate management of glyphosate on GMHT maize and for the development of weed resistance management strategies in each Member State permitting the use of glyphosate on maize GA21. It is necessary to exploit properly the appropriate antiresistance strategy to avoid undue herbicide usage*" (section 7.6.2 of the environmental risk assessment report of the CZ CA). The CZ CA recommended the applicant "*to design appropriately a relevant Technical User Guide for farmers that should involve Good Agricultural Practices of glyphosate applications guaranteeing sustainable and safe use of the entire GA21 technology*" (section 9 of the environmental risk assessment report of the CZ CA).

The EFSA GMO Panel gave its opinion and made recommendations on the scientific quality of the post-market environmental monitoring plan proposed by the applicant. In order to assess the efficacy of risk mitigation measures put in place to reduce levels of risk and in order to reduce the remaining scientific uncertainty identified in the environmental risk assessment, the EFSA GMO Panel recommends case-specific monitoring, as detailed above, to address (1) changes in botanical diversity within fields due to novel herbicide regimes, and (2) resistance evolution to glyphosate in weeds due to novel herbicide regimes. The EFSA GMO Panel considers that risk managers should adapt monitoring methodologies to their local receiving environments, management systems and the interplay between the legislation for GMOs and plant protection products.

The EFSA GMO Panel agrees with the general surveillance plan for the cultivation of maize GA21 of the applicant (1) to establish farmer questionnaires as a reporting format of any on-farm observations of effects associated with the cultivation of maize GA21, (2) to use existing monitoring networks which observe changes in biota and production practices from farm up to regional level to obtain data on environmental impacts in the landscape where maize GA21 is cultivated, (3) to review all new scientific, technical and other information pertaining to maize GA21, and (4) to develop stewardship programs for the introduction, marketing, management and stewardship of maize GA21, but requests that its proposals and those made by the CZ CA to strengthen general surveillance are implemented. The EFSA GMO Panel agrees with the reporting intervals and modalities proposed by the applicant. The general surveillance plan for the import and processing of maize GA21 was previously evaluated by the EFSA GMO Panel (EFSA, 2007).

The CZ CA considered that "*case-specific monitoring is the most appropriate way to monitor identified potential environmental effects that could result from changes in the technology. Therefore it is proposed that the applicant performs a case-specific monitoring of long-term effects with focus on non-target organisms, weed shifts, the development of herbicide resistance to glyphosate and microbial biodiversity*" (section 8.1 of the environmental risk assessment report of the CZ CA).

The CZ CA considered that "*the general surveillance plan should be updated*" and made specific proposals to strengthen general surveillance (section 8.2 of the environmental risk assessment report of the CZ CA).

In conclusion, the EFSA GMO Panel considers that the information available for maize GA21 addresses the scientific comments raised by Member States and that maize GA21, as described in this application, is as safe as its conventional counterpart and commercial maize varieties with respect to potential adverse effects on human and animal health, in the context of its intended uses. The EFSA GMO Panel also concludes that maize GA21 is unlikely to raise additional environmental safety concerns compared to conventional maize, but that its cultivation management could result in environmental harm under certain conditions. The EFSA GMO Panel therefore recommends managing the use of glyphosate on maize GA21 within diversified cropping regimes that have similar or reduced environmental impacts compared with conventional maize cultivation. The EFSA GMO Panel advises the deployment of case-specific monitoring to address (1) changes in botanical diversity within fields due to novel herbicide regimes, and (2) resistance evolution to glyphosate in weeds due to novel herbicide regimes. If subject to appropriate management measures, the cultivation management of maize GA21 is unlikely to raise safety concerns for the environment.

DOCUMENTATION PROVIDED TO EFSA

1. Letter from the Competent Authority of the United Kingdom, dated 16 July 2008, concerning a request for placing on the market of maize GA21 in accordance with Regulation (EC) No 1829/2003.
2. Acknowledgement letter, dated 30 July 2008, from EFSA to the Competent Authority of the United Kingdom.
3. Letter from EFSA to the applicant, dated 26 August 2008, requesting additional information under completeness check of the application.
4. Letter from the applicant to EFSA, received 12 September 2008, providing the additional information requested by EFSA under completeness check of the application.
5. Letter from EFSA to the applicant, dated 21 October 2008, delivering the ‘Statement of Validity’ for application EFSA-GMO-UK-2008-60 (placing on the market of maize GA21) submitted by Syngenta Seeds under Regulation (EC) No 1829/2003.
6. Letter from EFSA (CZ CA) to the applicant, dated 3 February 2009, requesting additional information and stopping the clock.
7. Letter from the applicant to EFSA, received 6 April 2009, providing the timeline for submission of the response.
8. Letter from the applicant to EFSA (CZ CA), received 13 July 2009, providing additional information.
9. Letter from EFSA (CZ CA) to the applicant, dated 11 September 2009, requesting additional information and maintaining the clock stopped.
10. Letter from the applicant to EFSA (CZ CA), received 27 October 2009, providing additional information.
11. Letter from EFSA (CZ CA) to the applicant, dated 21 December 2009, restarting the clock.
12. Letter from EFSA to the applicant, dated 12 January 2010, requesting additional information and stopping the clock.
13. Letter from the applicant to EFSA, received 26 January 2010, spontaneously providing clarifications.
14. Letter from the applicant to EFSA, received 17 February 2010, providing additional information.
15. Letter from EFSA (CZ CA) to the applicant, dated 10 May 2010, maintaining the clock stopped.

16. Letter from EFSA (CZ CA) to the applicant, dated 7 June 2010, requesting additional information and maintaining the clock stopped.
17. Letter from the applicant to EFSA (CZ CA), received 21 July 2010, providing additional information.
18. Letter from EFSA (CZ CA) to the applicant, dated 19 October 2010, restarting the clock.
19. Letter from EFSA to the applicant, dated 26 October 2010, requesting additional information and stopping the clock.
20. Letter from the applicant to EFSA, received 3 January 2011, providing additional information.
21. Letter from EFSA to the applicant, dated 18 April 2011 requesting additional information and maintaining the clock stopped.
22. Letter from the applicant to EFSA, received 29 April 2011, providing additional information spontaneously.
23. Letter from EFSA to the applicant, dated 3 May 2011, requesting additional information and maintaining the clock stopped.
24. Letter from the applicant to EFSA, received 30 May 2011, providing additional information and a timeline for submission of responses.
25. Letter from the applicant to EFSA, received 3 August 2011, changing the timeline for submission of responses.
26. Letter from the applicant to EFSA, received 4 October 2011, providing additional information.
27. Letter from the applicant to EFSA, received 16 November 2011, providing additional information spontaneously.
28. Letter from the applicant to EFSA, received 28 November 2011, providing additional information spontaneously.
29. Letter from EFSA to the applicant, dated 29 November 2011, restarting the clock.

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