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SCIENTIFIC OPINION

Applications (references EFSA-GMO-NL-2005-22, EFSA-GMO-RX-NK603) for the placing on the market of the genetically modified glyphosate tolerant maize NK603 for cultivation, food and feed uses, import and processing and for renewal of the authorisation of maize NK603 as existing products, both under Regulation (EC) No 1829/2003 from Monsanto[1]

Scientific Opinion of the Panel on Genetically Modified Organisms

(Questions No EFSA-Q-2005-249, No EFSA-Q-2008-075)

Adopted on 27 May 2009

PANEL MEMBERS[*]

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SUMMARY

This document provides a scientific opinion of the Panel on Genetically Modified Organisms (GMO Panel) of the European Food Safety Authority (EFSA) on two applications (References EFSA-GMO-NL-2005-22 and EFSA-GMO-RX-NK603) submitted by Monsanto under Regulation (EC) No 1829/2003 for (1) the placing on the market of the genetically modified (GM) glyphosate tolerant maize NK603 for cultivation, food and feed uses and import and processing, as well as for (2) the renewal of the authorisation of existing products produced from GM maize NK603 (Unique Identifier MON-ØØ6Ø3-6).

The scope of these two applications covers:

- cultivation, food and feed uses and import and processing (Reference EFSA-GMO-NL-2005-22);

[1] For citation purposes: Scientific Opinion of the Panel on Genetically Modified Organisms on applications (EFSA-GMO-NL-2005-22 and EFSA-GMO-RX-NK603) for the placing on the market of the genetically modified glyphosate tolerant maize NK603 for cultivation, food and feed uses and import and processing, and for renewal of the authorisation of maize NK603 as existing product. *The EFSA Journal* (2009) 1137, 1-50.

[*] (minority opinion) This opinion is not shared by 0 members of the Panel. / (conflict of interest) 0 members of the Panel did not participate in (part of) the discussion on the subject referred to above because of possible conflicts of interest.

- the continued marketing of existing food additives and feed (feed materials and feed additives) produced from maize NK603 which were lawfully placed on the market in the Community before the date of entry into force of Regulation (EC) No 1829/2003 (Reference EFSA-GMO-RX-NK603). After the date of entry into force of Regulation (EC) No 1829/2003, these products were notified to the European Commission according to Articles 8 and 20 of that Regulation and included in the Community Register of genetically modified food and feed[2].

Maize NK603 has been developed for tolerance to glyphosate (also refer to as GMHT crop) by the introduction, via particle gun acceleration, of a gene coding for 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) from *Agrobacterium* sp. strain CP4 (CP4 EPSPS).

In delivering its scientific opinion, the EFSA GMO Panel considered the applications EFSA-GMO-NL-2005-22 and EFSA-GMO-RX-NK603, additional information supplied by the applicant, the scientific comments submitted by Member States and the report of the Spanish Competent Authority and its Biosafety Commission.

The EFSA GMO Panel assessed maize NK603 with reference to the intended uses and appropriate principles described in the guidance document of the EFSA GMO Panel for the risk assessment of GM plants and derived food and feed. The scientific assessment included molecular characterisation of the inserted DNA and expression of target proteins. A comparative analysis of agronomic traits and composition was undertaken, and the safety of the new protein and the whole food/feed were evaluated with respect to potential toxicity, allergenicity and nutritional quality. An assessment of environmental impacts and the post-market environmental monitoring plan were undertaken.

Data for molecular characterisation established that the insert is a single complete copy of the plasmid vector fragment used (PV-ZMGT32L) and that there is no detectable presence of plasmid DNA from outside of this fragment. Appropriate analyses of the integration site, including sequence determination of the inserted DNA and flanking regions and bioinformatic analysis, have been performed. Bioinformatic analysis of junction regions demonstrated the absence of any potential new ORFs coding for known toxins or allergens. The expression of the new proteins (CP4 EPSPS and CP4 EPSPS L214P) produced by the genetic modification has been sufficiently analysed and the stability of the genetic modification has been demonstrated over several generations. Variations in protein levels were observed in field trials but given the fact that the CP4 EPSPS proteins are demonstrated to be safe, this does not raise any safety concern. The EFSA GMO Panel is therefore of the opinion that the molecular data provided are sufficient and do not raise a safety concern.

Based on the results of compositional analysis of grain and forage material of maize NK603 collected at field trials from a representative range of environments and seasons, the EFSA GMO Panel concludes that maize NK603 is compositionally equivalent to conventional maize, except for the presence of the CP4 EPSPS proteins. In addition, field trials did not show changes in phenotypic characteristics and agronomic performance except for the introduced trait.

[2] http://ec.europa.eu/food/dyna/gm_register/gm_register_auth.cfm?pr_id=11

There were no adverse effects in a 90-day feeding study on rats with NK603 maize grain. Feeding studies on broiler chickens, Angus-continental cross steers, Holstein dairy cows, growing-finishing pigs, and rats provided evidence of nutritional equivalence of maize NK603 to conventional maize. In addition, there is no evidence that the overall allergenicity of the whole plant is changed. The EFSA GMO Panel is of the opinion that maize NK603 is as safe as conventional maize. Maize NK603 and derived products are unlikely to have any adverse effect on human and animal health in the context of the intended uses.

The Spanish Competent Authority and its Biosafety Commission provided to EFSA its opinion on the environmental risk assessment in line with Articles 6.3 (e) and 18.3 (e) of Regulation (EC) No 1829/2003. The Spanish Competent Authority and its Biosafety Commission conclude that "*according to the current state of scientific knowledge and after examining the existing information and data provided by the Monsanto Company, the Spanish Commission on Biosafety could give a favourable opinion to the commercialisation in the E.U. of maize NK603 if proposals and conditions established in the ERA report are implemented*".

The EFSA GMO Panel considers that maize NK603 has no altered survival, multiplication or dissemination characteristics and interacts with other organisms as conventional maize. The likelihood of unintended environmental effects due to the establishment and spread of maize NK603 will be no different from that of traditionally bred maize. The EFSA GMO Panel considers that the potential environmental impacts of the specific cultivation, management and harvesting techniques of maize NK603 are indirect effects entirely associated with the use of the complimentary herbicide regimes. Thus the EFSA GMO Panel concludes that maize NK603 plants are unlikely to cause any direct adverse effects, but that potential adverse environmental effects of the cultivation of maize NK603 associated with the use of the complimentary glyphosate herbicide have been identified. This conclusion is in line with the conclusions of the Spanish Competent Authority and its Biosafety Commission.

The EFSA GMO Panel recommends that the potential adverse effects of the glyphosate should be evaluated for the specific use on maize NK603 during the national registration by Member States under the pesticide Directive 91/414/EEC. In addition, the EFSA GMO Panel recommends that the occurrence of weed resistance and appropriate management strategies should be addressed as part of the registration of glyphosate under Directive 91/414/EEC. In line with its interplay working document (EFSA, 2008) and the requirements of Directive 2001/18/EC (EC, 2001), the EFSA GMO Panel also recommends glyphosate use on maize NK603 in regimes that have similar or reduced environmental impacts compared with conventional maize cultivation. The Spanish Competent Authority and its Biosafety Commission propose that monitoring should be conducted under Directive 2001/18/EC and recommend to "*consider deeper studies on the following potential adverse effects: the potential indirect effects on non-target organisms due to the weed management, the development of weed resistance to glyphosate and the evolution of the flora associated to management of the cultivation of NK603 maize and their potential impacts on biodiversity*". However, the EFSA GMO Panel is of the opinion that an alternative option would be the use of herbicide management measures in conjunction with the monitoring for weed resistance evolution under Directive 91/414/EEC (as proposed by the Spanish Competent Authority and its Biosafety Commission) and general surveillance of maize NK603 under Directive 2001/18/EC to detect unanticipated adverse effects.

The EFSA GMO Panel agrees with the general methods and approaches of the general surveillance plan, but advises the applicant to describe in more detail how information will be collected that could be used to assess whether the intended uses of maize NK603 and its specific management are having unanticipated adverse environmental effects.

In conclusion, the EFSA GMO Panel considers that the information available for maize NK603 addresses the scientific comments raised by Member States and that maize NK603 is as safe as its conventional counterpart with respect to potential direct effects on human and animal health and the environment. However, the EFSA GMO Panel concludes that the cultivation management of maize NK603 could have adverse effects on the environment in the context of its intended uses. The EFSA GMO Panel therefore recommends managing the use of glyphosate on maize NK603 in regimes that have similar or reduced environmental impacts compared with conventional maize cultivation.

Key words: **GMO, maize (*Zea mays*), NK603, herbicide tolerant, glyphosate, cultivation, food and feed uses, food safety, feed safety, human and animal health, environment, Regulation (EC) No 1829/2003, Directive 2001/18/EC, renewal, existing products**

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BACKGROUND

On 5 October 2005, the European Food Safety Authority (EFSA) received from the Competent Authority of the Netherlands an application (Reference EFSA-GMO-NL-2005-22), for authorisation of the genetically modified (GM) glyphosate tolerant maize NK603 (Unique Identifier MON-ØØ6Ø3-6) submitted by Monsanto under Regulation (EC) No 1829/2003 (EC, 2003) for cultivation, food and feed uses and import and processing.

The applicant has submitted this application jointly with an application for renewal of the authorisation of existing feed materials and food and feed additives produced from maize NK603, notified as existing products under Regulation (EC) No 1829/2003. In agreement with the European Commission (letter SANCO D4/SG/cc-D(05)440797 received 4 October 2005), it has been decided that the new and renewal application would be assessed together by the EFSA Panel on Genetically Modified Organisms (EFSA GMO Panel).

The European Commission has granted the following authorisation for maize NK603:

- The Commission Decision of 19 July 2004 concerning the placing on the market, in accordance with Directive 2001/18/EC of the European Parliament and of the Council, of a maize product (*Zea mays* L. line NK603) genetically modified for glyphosate tolerance, to be used as any other maize, with the exception of cultivation and uses as or in food. The Spanish Competent Authority was the lead country in the assessment of the respective notification (Notification C/ES/00/01).

- The Commission Decision (2005/448/EC) of 3 March 2005, authorising the placing on the market of foods and food ingredients derived from genetically modified line maize NK603 as novel foods or novel food ingredients, under Regulation (EC) No 258/97 of the European Parliament and of the Council.

The EFSA GMO Panel has previously issued scientific opinions on GM plant market authorisation applications of maize NK603 (EFSA 2003a,b). In addition, several applications concerning stacked transformation events including event NK603 (maize 59122 x 1507 x NK603, 59122 x NK603, NK603 x MON810, MON863 x MON810 x NK603, MON863 x NK603, 1507 x NK603, NK603 x MON810) for food and feed uses, import and processing have been assessed[3].

After receiving both applications and in accordance with Articles 5(2)(b) and 17(2)b of Regulation (EC) No 1829/2003, EFSA informed Member States as well as the European Commission and made the summary of these applications publicly available on the EFSA website. EFSA initiated a formal review of both applications to check compliance with the requirements laid down in Articles 5(3) and 17(3) of Regulation (EC) No 1829/2003. On 25 April 2006, EFSA received additional information requested under completeness check (requested on 23 March 2006) and on 12 May 2006 EFSA declared both applications as valid in accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003.

On 19 December 2005, 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 Spanish Competent Authority to conduct the initial

[3] List of applications received and finalised are available: http://registerofquestions.efsa.europa.eu/roqFrontend/questionsListLoader?panel=ALL

environmental risk assessment of application EFSA-GMO-NL-2005-22 concerning the placing on the market of maize NK603 for cultivation.

EFSA made the valid applications 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 (EC, 2001) following the requirements of Articles 6(4) and 18(4) of Regulation (EC) No 1829/2003, to request their scientific opinion. The Member State bodies had three months after the date of receipt of the valid applications (until 11 August 2006) within which to make their opinion known.

The Spanish Competent Authority asked the applicant for additional data on maize NK603 on 22 September 2006, 15 February 2007 and 19 November 2007. The applicant provided the requested information on 15 December 2006, 10 October 2007 and 11 December 2007.

The EFSA GMO Panel asked the applicant for additional data on maize NK603 on 8 February 2007, 28 April 2008, 7 November 2008 and 16 February 2009. The applicant provided the requested information on 8 August 2007, 1 October 2008, 15 December 2008 and 25 February 2009. After receipt and assessment of the full data package, the EFSA GMO Panel finalised its risk assessment of maize NK603.

The EFSA GMO Panel carried out a scientific assessment of the maize NK603 for cultivation, food and feed uses and import and processing in accordance with Articles 6(6) and 18(6) of Regulation (EC) No 1829/2003, taking into consideration the scientific comments of Member States, the additional information provided by the applicant and the environmental risk assessment report from the Spanish Competent Authority and its Biosafety Commission.

In giving its opinion on maize NK603 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 receipt of the valid application. As additional information was requested by the Spanish Competent Authority and the EFSA GMO Panel, the time-limit of 6 months was extended accordingly, in line with Articles 6(1), 6(2), 18(1), and 18(2) of Regulation (EC) No 1829/2003.

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

TERMS OF REFERENCE

The EFSA GMO Panel was requested to issue a scientific opinion of (1) maize NK603 for cultivation, food and feed uses and import and processing in accordance with Articles 6(6) and 18(6) of Regulation (EC) No 1829/2003; and (2) for the renewal of the authorisation of existing products produced from maize NK603, that were previously notified according to Articles 8(1)(b) and 20(1)(b) of Regulation (EC) No 1829/2003 and that have now been submitted under Articles 8(4) and 20(4) of Regulation (EC) No 1829/2003.

Where applicable, any conditions or restrictions which should be imposed on the placing on the market and/or specific conditions or restrictions for use and handling, including post-

market monitoring requirements based on the outcome of the risk assessment and, in the case of GMOs or food/feed containing or consisting of GMOs, conditions for the protection of particular ecosystems/environments 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 an opinion on information required under Annex II to 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 GMO risk management.

ACKNOWLEDGEMENTS

The European Food Safety Authority wishes to thank the members of the Working Groups on Molecular Characterisation, Food/Feed and Environment, the Spanish Competent Authority and its Biosafety Commission, as well as the *ad hoc* expert Sue Hartley and the following members of its staff: Anna Christodoulidou, Yann Devos, Ana Gomes and Karine Lheureux for the preparation of this opinion.

ASSESSMENT

1. Introduction

Maize NK603 (Unique Identifier MON-ØØ6Ø3-6) is assessed with reference to its intended uses and the appropriate principles described in the guidance document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of GM plants and derived food and feed (EFSA, 2006a).

Maize NK603 has been developed for tolerance to glyphosate by the introduction, via particle gun acceleration, of a gene coding for 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) from *Agrobacterium* sp. strain CP4 (CP4 EPSPS).

2. Issues raised by Member States

Issues raised by Member States are addressed in Annex G of the EFSA overall opinion[4].

3. Molecular characterisation

3.1. Evaluation of relevant scientific data

3.1.1. Transformation process and vector constructs

Proprietary embryogenic maize cell culture AW x CW was the initial recipient of the introduced DNA by transformation using particle acceleration technology to develop the maize NK603 event.

Conventional breeding methods were used to backcross plants generated from the initial transformation into a recurrent, desired inbred maize line with a genetic background of interest to the breeder.

NK603 has been developed for tolerance to glyphosate by the introduction of a gene coding for glyphosate tolerant 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) from *Agrobacterium* sp. strain CP4 (CP4 EPSPS). Particle acceleration was used to introduce a fragment of DNA isolated from the bacterial plasmid vector PV-ZMGT32. The plasmid vector contains two adjacent plant gene expression cassettes each containing a single copy of the CP4 *epsps* gene fused to chloroplast transit peptide (CTP) sequences. CTP targets the CP4 EPSPS protein to its natural sub-cellular location in the chloroplast. In the first *ctp*2-CP4 *epsps* cassette the coding sequence is regulated by the rice actin promoter and a rice intron sequence introduced upstream of the *ctp* sequence. Expression of the second *ctp*2-CP4 *epsps* cassette is regulated by an enhanced 35S CaMV promoter and a maize intron derived from a gene encoding a heat shock protein. In each cassette the CP4 *epsps* sequence is linked to the nopaline synthase terminator (NOS 3') sequence from *Agrobacterium tumefaciens*. The vector also contains an *npt*II bacterial selectable marker gene (for kanamycin resistance; derived from the prokaryotic transposon Tn5) and an origin of replication (*ori*). A *Mlu*I restriction fragment of the PV-ZMGT32 plasmid vector, designated PV-ZMGT32L, was used

[4] http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2005-249
http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2005-075

for transformation and this fragment only contains the CP4 *epsps* plant gene expression cassettes. The *npt*II gene as well as the *ori* are not present in the fragment PV-ZMGT32L.

3.1.2. Transgenic constructs in the genetically modified plant

Southern analysis, PCR and DNA sequencing have been used to provide data on inserts within the derived GM event NK603. The analysis included the use of appropriate restriction endonucleases and showed that the insert is a single complete copy of PV-ZMGT32L. There is no detectable presence of plasmid DNA from outside of the vector fragment PV-ZMGT32L.

Molecular data revealed that both *ctp*2-CP4 *epsps* gene cassettes are intact within NK603. The sequence of the CP4 *epsps* gene from the first cassette in NK603 is identical to that in the original plasmid, whilst in the second inserted cassette the sequence of the CP4 *epsps* gene differs by two nucleotides from that in the original plasmid. These nucleotide changes result in one silent mutation (i.e. no amino acid modification) and one amino acid substitution of proline for leucine at amino acid position 214 (hence the gene is designated CP4 *epsps l214p*).

The insert also includes at the 3' end an additional 217 bp DNA fragment of the rice actin promoter. This fragment does not contain sequences needed for promoter activity. Next to this 217 bp fragment is a 305 bp region with homology to chloroplast DNA. Further sequencing of 5' (307 bp) and 3' (497 bp) flanking regions confirmed the sequences to be maize genomic DNA. Bioinformatics analysis did not indicate any interruption of known maize genes.

Bioinformatic analyses were carried out to assess the potential toxicity, allergenicity or pharmacological activity of putative polypeptides encoded at the 5' and 3' junctions of a segment of chloroplast DNA that is located downstream of the 3' end of the NK603 insertion event. Putative polypeptides 5' to the NK603 insert were defined by the junction of a segment of the rice actin promoter and the chloroplast DNA. Polypeptides 3' to the insert were defined by the junction of the chloroplast DNA and plant genomic DNA. Similarly, a bioinformatics assessment was carried out on the potential toxicity, allergenicity or pharmacological activity of putative polypeptides, defined by the 5' junction of the NK603 insert and plant genomic DNA.

The results of these 3' and 5' end bioinformatic analyses, which were updated in 2008, demonstrate that in the highly unlikely event that any of the junction polypeptides were translated, they do not share sequence similarity or identity to known toxic or allergenic proteins

In addition to the analyses described above, reverse transcriptase PCR (RT-PCR) analyses were conducted across the 3' junction between the insert in maize event NK603 and the adjacent maize genomic DNA sequences. The RT-PCR data demonstrated that there was no detectable transcription into the NK603 insert from the maize genomic DNA sequence flanking the 3' end of the inserted DNA. However, the data did demonstrate that an RNA species could be detected that likely initiated in the promoter of the NK603 insert and proceeded through the *nos 3'* transcriptional termination sequence continuing into the maize genomic DNA flanking the 3' end of the insert. Northern analyses with probes directed at potentially transcribed sequences downstream of the *nos 3'* element failed to detect the larger read-through transcript. Moreover, when these same blots were probed with a segment of the

CP4 *epsps* coding region, only one band of 1.4 kb, the expected size of the CP4 *epsps* transcript terminating within the NOS terminator, was observed. This suggests that the read-through transcript accumulates at very low levels only detectable by a highly sensitive method such as RT-PCR.

As indicated above, in the highly unlikely event that any of the junction polypeptides were translated, bioinformatic analyses revealed that they would not share sequence similarity or identity to known toxins or allergens.

3.1.3. Information on the expression of the insert

The levels of CP4 EPSPS and CP4 EPSPS L214P proteins in various tissues of maize NK603, produced during the 1999 growing season in Europe and the 2002 growing season in the USA, were estimated using an enzyme-linked immunosorbent assay (ELISA). The values provided were for the sum of both CP4 EPSPS and CP4 EPSPS L214P proteins, as the ELISA recognises both proteins in NK603. Both proteins are referred to as CP4 EPSPS in this section. In maize forage, the mean CP4 EPSPS protein levels from the four different field sites in Europe were as follows: 44.2 µg/g fw (fresh weight) (site 1, Southern France), 45.7 µg/g fw (site 2, Southern France), 43.6 µg/g fw (site 3, Northern France), and 60.9 µg/g fw (site 4, Italy). The overall mean CP4 EPSPS protein level in maize forage across all four sites was 48.6 µg/g fw. In maize grain, the respective values for the 4 sites were 13.2 µg/g fw, 12.7 µg/g fw, 2.2 µg/g fw and 5.5 µg/g fw. The overall mean CP4 EPSPS protein level in maize grain across all four sites was 8.4 µg/g fw.

In 2002, NK603 samples were produced in field trials in the USA (Iowa, Missouri, Ohio and Nebraska). These field sites were located within major maize growing region of the USA and provided a variety of environmental conditions. At each site, three replicate plots containing NK603 and the non-transgenic control were planted using a randomized complete block design. Leaf, pollen, forage, forage root and grain tissues were collected over the growing season (4 time points) from each replicated plot at all field sites. Over the harvest periods selected the ranges of CP4 EPSPS protein levels across four field sites for leaf tissues were 49 to 160 µg/g fw, for root 5.8 to 31 µg/g fw, for forage 15 to 52 µg/g fw, for forage root 12 to 33 µg/g fw, for pollen 250 to 460 µg/g fw, and for grain 7.5 to 16 µg/g fw. The expression levels for forage and grain are in general agreement with the CP4 EPSPS levels measured in forage and grain samples collected from six non-replicated and two replicated field trials conducted in 1998 in the USA, which were previously reported in Monsanto's notification C/ES/00/01 under Directive 2001/18/EC. In the USA trials from 1998, CP4 EPSPS expression levels ranged from 18.0 to 31.2 µg/g fw for forage and from 6.9 to 15.6 µg/g fw for grain samples, respectively.

3.1.4. Inheritance and stability of inserted DNA

Southern analysis was undertaken to confirm the genetic stability of the inserted DNA in maize NK603 using *Eco*RV digests probed with the full-length *ctp2*-CP4 *epsps* fragment. Segregation data for nine generations are provided including six generations of crossing and three generations of self pollination.

In the Southern analysis, no differences in banding pattern were observed for the generations tested, demonstrating the stability of the inserted DNA. This is consistent with a single site of

integration into the genomic DNA of NK603. In further studies, the stability of the introduced DNA was confirmed in seven generations representing five separate breeding lines derived from the original transformation event. Stability of the glyphosate tolerance trait was also confirmed over nine generations.

3.2. Conclusion

The glyphosate-tolerant maize NK603 contains a single copy insert proven to be stable in inheritance studies. There are no additional vector sequences present and sequence information on the insert and flanking regions combined with bioinformatic analysis indicates that in the unlikely event a novel fusion protein were to be produced it would have no similarities to known allergens or toxins. Given the fact that the CP4 EPSPS proteins are demonstrated to be safe, variations in protein levels observed in field trials do not raise any safety concern. The EFSA GMO Panel is therefore of the opinion that the molecular data provided are sufficient and do not raise a safety concern.

4. Comparative analysis

4.1. Evaluation of relevant scientific data

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

The applicant confirms that LH82 x B73 (sometimes abbreviated "B73") is the non-GM counterpart with a comparable genetic background used in the molecular characterisation of transformation event NK603 in maize, the field trials performed to collect material for compositional analyses, and the safety studies. Furthermore, molecular studies of the flanking regions of the genes inserted in maize event NK603 established that these are related to the genomic DNA of the B73 control maize and are native to the maize genome. LH82 x B73 has a genetic background comparable to that of maize NK603 and does not express the CP4 EPSPS proteins present in maize NK603. Thus LH82 x B73 (B73) can be considered as an appropriate control line for comparative assessments.

Grain and forage samples from field trials in the USA in 1998 and in Europe in 1999 were collected for the analysis of chemical composition. The US field trials in 1998 were conducted in Iowa, Illinois, Indiana, and Ohio at two replicated and six non-replicated trial sites. The field trials in Europe in 1999 were carried out in France and Italy at in total four replicated trial sites. Whereas maize NK603 was treated twice with glyphosate herbicide during the field trials in the US and once in Europe, the non-modified LH82 x B73 control maize was treated with conventional herbicides.

4.1.2. Compositional analysis

The EFSA GMO Panel has already assessed the composition of maize NK603 relative to its non-GM counterpart maize LH82 x B73 when giving its opinion on applications/notifications to place maize NK603 on the market for import and processing, or for supplying food and feed ingredients. A summary of the compositional data in these applications/notifications is available in the open literature (Ridley et al., 2002). The EFSA GMO Panel concluded that

maize NK603 can be considered to have the same composition as the genetically related control maize (EFSA, 2003a,b).

In short, the composition of maize NK603 and the non-GM control maize was compared with regard to 44 parameters in grain and 7 in forage. The analysed parameters in grain were ash, carbohydrates, fibre, moisture, protein, total fat, amino- and fatty acids, minerals (Ca, Cu, Fe, K, Mg, Mn, Na, P, Zn), vitamin E, phytic acid and trypsin inhibitor, whereas forage was analysed for proximates and neutral and acid detergent fibre. The levels of the various constituents in maize NK603 were either comparable to the levels found in the non-modified control or within the ranges reported for the respective constituents in conventional maize varieties. The provided baseline compositional data were either compiled from data in the public literature, or compiled from data of previous studies on the composition of maize cultivated by the Monsanto Company between 1993 and 1995 (historical data).

Statistical analysis of the field trial data revealed one significant difference in 1998, for stearic acid in grain. This difference was minor (NK603: 1.95% of total fatty acids; control: 1.86%) and was not observed in 1999. In the material collected in Europe in 1999, the statistically significant differences between maize NK603 and the non-GM control were observed only at one or a few of the four trial site. Five of the statistically significant differences identified were assessed in more detail (phosphorus, leucine, zinc, protein and carbohydrate levels in grain). As differences were modest, were not observed at other trial sites, and levels were within the range identified in conventional maize varieties and reported in the literature, also these statistical differences were not considered biologically relevant. The biological relevance of the statistical differences was further assessed by performing additional comparisons of the level of these compounds in maize NK603 and conventional non-GM maize lines grown in field trials conducted in 1994-1995 or 1998. No conclusive differences requiring further studies were found. Thus maize NK603 used for food, feed and processing is considered to have the same composition as the genetically related non-GM maize.

4.1.3. Agronomic traits and GM phenotype

Field trials performed according to Good Experimental Practices and European and Mediterranean Plant Protection Organization guidelines at a total of nine locations in Germany and France between 2000 and 2002 were used for the comparative assessment of phenotypic and agronomic characteristics of maize NK603 varieties and their appropriate non-modified control maize varieties. These trials aimed at studying parameters of plant growth and development, yield, plant and ear morphology, and plant health and pest susceptibility (including susceptibility to pests, diseases, and applied pesticides). These investigations showed that, with the exception of glyphosate tolerance, maize NK603 is phenotypically and agronomically equivalent to the non-GM counterpart and to conventional maize varieties.

4.2. Conclusion

The comparative analysis of maize NK603 to an appropriate non-GM maize variety with a comparable genetic background and to other conventional maize varieties provided evidence that these maize varieties are compositionally and agronomically equivalent, except for the presence of the CP4 EPSPS and CP4 EPSPS L214P proteins in maize NK603, and that no unintended effects have appeared as a result of the genetic modification.

5. Food/Feed safety assessment

The food and feed safety of maize NK603 has already been assess by the EFSA GMO Panel in connection with delivering its opinion on previous applications for placing on the market maize NK603, for import and processing, under Directive 2001/18/EC, and for foods and food ingredients derived from maize NK603, under Regulation (EC) No 258/97 (EFSA, 2003a,b).

5.1. Evaluation of relevant scientific data

5.1.1. Product description and intended use

The scope of the present applications includes use of maize NK603 for food or feed, food or feed containing or consisting of this maize, food or feed produced from or containing ingredients produced from this maize, as well as for cultivation.

Maize NK603 has been genetically modified to express of the CP4 EPSPS and CP4 EPSPS L214P proteins. This modification is intended to improve the agronomic performance only and is not intended to influence the nutritional properties, the processing characteristics and overall use of maize as a crop. The primary use of maize is for animal feed, but it is also processed into valuable food products, including e.g. starch, syrups, ethanol and oils.

5.1.2. Effect of processing

Since maize NK603 has been found to be compositionally equivalent to the control maize and commercial maize hybrids, except for the newly expressed trait (see section 3.2.2), the effect of processing on the constituents of maize NK603 is not expected to be different compared to that on conventional maize.

A multitude of processes are used in maize processing, including temperature treatments, hydrolyses, soaking in slightly acidic water, and drying. Any of these methods are likely to influence degradation and/or denaturation of constituents but there is no indication that they will influence maize NK603 differently than conventional maize. To demonstrate the influence of processing on the CP4 EPSPS enzyme derived from a recombinant *Escherichia coli* strain (see section 4.2.3.1), the applicant studied the influence of heat (25-75^{o}C for 15 or 30 minutes) on the specific activity of the CP4 EPSPS enzyme. Temperatures up to 45°C had no or only a slight influence on enzymatic activity, incubation of the enzyme at 55°C for 15 minutes reduced the activity to less than half of that observed after incubation at 25°C, whereas higher temperatures (65 and 75°C) completely inactivated the enzyme. In other incubation studies (for 15 minutes) at various pH values (pH 4-11), it was observed, when assaying enzyme stability and activity, that although there is a trend towards slightly lower activity at the low end of the pH range, the majority of the enzymatic activity is not irreversibly lost at either the low or high pH range. Considering the toxicological profile and allergenic properties of the CP4 EPSPS proteins (see sections 5.2.3 - 5.2.5), the EFSA GMO Panel is of the opinion that no further studies on effects of processing is required.

5.1.3. Toxicology

5.1.3.1. CP4 EPSPS protein used for safety assessment

Due to the comparatively low expression level of the CP4 EPSPS proteins in maize NK603 and the difficulty in isolating a sufficient quantity of purified protein from the genetically modified maize plant, the safety studies with the newly expressed proteins were conducted with a CP4 EPSPS protein encoded by the *cp4 epsps* gene from *Agrobacterium* sp. strain CP4 and a variant CP4 EPSPS L214P protein encoded by the *cp4 epsps l214p* gene, both having been expressed in *Escherichia coli*. The proteins differ by a single amino acid, i.e. a leucine in position 214 of CP4 EPSPS has been exchanged for a proline in CP4 EPSPS L214P. The CP4 EPSPS and CP4 EPSPS L214P proteins expressed in *E. coli* were shown to be structurally and functionally equivalent. Evidence provided included (1) modelling of CP4 EPSPS L214P protein structure (which showed that the amino acid substitution does not alter the predicted secondary and tertiary structure of the protein); (2) the high variability in known EPSPS proteins of the CP4 EPSPS protein domain containing the proline, and (3) demonstration of equivalent enzyme activities for CP4 EPSPS and CP4 EPSPS L214P proteins. The structural similarity and physico-chemical and functional equivalence of the CP4 EPSPS proteins produced by *E. coli* to those produced in maize NK603 was shown by Western analysis, mobility in SDS-PAGE, MALDI-TOF mass spectrometry, glycosylation analysis and CP4 EPSPS enzymatic activity. All these methods confirmed the equivalence of the bacterial and the plant CP4 EPSPS proteins.

Based on the identified similarity in structure and equivalence in physico-chemical properties and function between these proteins, the EFSA GMO Panel accepts the use of CP4 EPSPS and CP4 EPSPS L214P test material derived from *E. coli* for the degradation studies and safety testing of the respective proteins present in maize NK603 and as a reference standard in the ELISA to estimate CP4 EPSPS expression levels in various tissues of maize NK603.

5.1.3.2. Toxicological assessment of expressed novel protein in maize NK603

As EPSPS enzymes occur in a wide range of plants and fungi, and in some microorganisms, humans have a long history of dietary exposure to these proteins. No adverse effects have been reported with their intake. Previous applications for placing on the market glyphosate resistant crops have included safety assessments of the CP4 EPSPS protein. It has been concluded that these GM plants and the CP4 EPSPS protein expressed are safe for human and/or animal consumption (SCP, 1998a,b; EFSA, 2004a, 2006c, 2008a). The EFSA GMO Panel is of the opinion that no scientific data have emerged which call for a change of this opinion.

(a) Acute toxicity testing

Acute oral toxicity studies were performed using mice. CP4 EPSPS and CP4 EPSPS L214P proteins produced in *Escherichia coli* were administered by gavage to CD-1 mice at single doses up to 572mg/kg body weight and 817mg/kg body weight, respectively, without indications of adverse effects.

(b) Degradation in simulated digestive fluids

Simulated gastric fluid (pH 1.2) containing pepsin and recombinant CP4 EPSPS or CP4 EPSPS L214P at a ratio 2.89:1 (w/w) was incubated for various length of time at 37°C and the simulated gastric fluid analysed for intactness of the CP4 EPSPS proteins. More than 95% of the proteins were digested within 15 seconds as demonstrated by colloidal blue staining and Western blotting of SDS-PAGE gels. In addition, digestion of the CP4 EPSPS L214P protein in simulated intestinal fluid at a pancreatin:CP4 EPSPS L214P ratio of 55:1 (w/w; buffer pH 7.5) was studied. More than 90% of the CP4 EPSPS L214P protein was digested within 4 hours at 37°C as demonstrated by Western blotting of SDS-PAGE gels. These results confirm previous findings and show that the recombinant CP4 EPSPS enzymes were rapidly degraded in simulated gastric and intestinal fluid.

(c) Bioinformatic studies

The amino acid sequences of the two different CP4 EPSPS proteins were compared with the amino acid sequences of known toxic proteins using a bioinformatics approach . No relevant similarities between the sequence of the CP4 EPSPS proteins and sequences of toxic proteins were found.

(d) Utilisation of CP4 EPSPS-expressing crops worldwide

Maize NK603 has been commercially cultivated and marketed since 2001 in the US and Canada, and since 2004 in Argentina. In addition, genetically modified soybeans (GM 40-3-2 and MON89788), expressing the same CP4 EPSPS protein as maize NK603, have been used worldwide since 1996 and 2007. In 2008, these soybean varieties were cultivated on more than 65 million hectares and last years approximately 70% of all soybeans placed on the market contained the CP4 EPSPS protein (James, 2008).

5.1.3.3. Toxicological assessment of new constituents other than proteins

Since no new constituents other than the above mentioned CP4 EPSPS proteins are expressed in maize NK603, and there is no indication of alterations in levels of endogenous compounds, a toxicological assessment of new constituents is not applicable.

5.1.3.4. Toxicological assessment of the whole GM food/feed

A published 90-day study in rats fed either maize NK603 as 11% or 33% of the diet, or a diet which to 11% or 33% was made up of non-GM maize grain having a comparable genetic background to maize NK603 (LH82 x B73), resulted in no consistent differences in the measured clinical, biochemical and histological parameters, except for slightly elevated levels of average corpuscular volume and average corpuscular haemoglobin in female rats administered the high dose (Hammond et al., 2004). Since both parameters are calculated (hematocrit/red blood cells and haemoglobin concentration/red blood cells, respectively), and no other observations of treatment related effects were made, the applicant suggested that these statistical differences were artefacts resulting from a slightly higher hematocrit or haemoglobin concentration and slightly lower red blood cell count at this sampling. Furthermore, it was pointed out that the observed difference in average corpuscular volume and average corpuscular haemoglobin had no biological relevance. The EFSA GMO Panel finds the interpretation of the data acceptable. The EFSA GMO Panel also found the doses chosen for the study (11% or 33% of diet) appropriate, as they did not distort the nutritional

balance of the experimental animals. The standard rodent diet used by the test laboratory contains approximately 33% maize grain.

5.1.4. Allergenicity

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

5.1.4.1. Assessment of allergenicity of the newly expressed proteins

Early risk assessments of GM soybean expressing CP4 EPSPS were performed both by national Competent Authorities within the European Community ACNFP, 1994; EC, 1996). The EFSA GMO Panel completed a risk assessment for allergenicity of the CP4 EPSPS and CP4 EPSPS L214P proteins already during the pre-market safety assessment of previous applications related to import and processing of maize NK603 and to marketing of food and feed ingredients of maize NK603 (EFSA, 2003a,b). These assessments included establishment of absence of known allergenicity of the gene source, absence of sequence homology of the CP4 EPSPS and CP4 EPSPS L214P proteins with proteins associated with allergenicity and celiac disease, and rapid and extensive degradation of these proteins by proteolytic enzymes. The EFSA GMO Panel is not aware of any new information on allergenicity of these proteins which requires the earlier opinion to be changed. Nor is the EFSA GMO Panel aware of any new tests which produce more relevant or accurate information on possible allergenicity of the protein and which provide a higher guarantee of safety.

Based on the information available the EFSA GMO Panel considers it unlikely that the CP4 EPSPS and CP4 EPSPS L214P proteins expressed in maize NK603 are allergens.

5.1.4.2. Assessment of allergenicity of the whole GM plant

Rare cases of occupational allergy to maize dust or maize pollen allergy, have been reported. Food allergy to maize is rare (Moneret-Vautrin et al., 1998), but IgE-binding proteins have been identified in maize flour (Pastorello et al., 2000; Pasini et al., 2002). Allergy to maize is detected in a minor fraction of the population of atopic patients. In addition, most individuals with a positive skin prick test (SPT) or having IgE antibodies against maize were suffering 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 sensitization to maize proteins is very rare. The allergenicity of the whole crop could be increased as an unintended effect of the random insertion of the transgene in the genome of the recipient, for example through qualitative or quantitative modification of the pattern of expression of endogenous proteins. This issue does not appear to be a safety concern to the EFSA GMO Panel since maize is not considered a major allergenic food. A theoretically possible over-expression of any endogenous protein would be unlikely to alter the overall allergenicity of the whole maize NK603 plant.

5.1.5. Nutritional assessment of GM food/feed

A nutritional performance study using diets containing 57-63% grain of glyphosate-sprayed maize NK603 or conventional herbicide-treated non-GM maize with a comparable genetic background (LH82 x B73) was carried out with rapidly growing broiler chickens, which reach full size within approximately six weeks. Analysis of the diets showed that mycotoxin levels were low and herbicide residue levels were below the maximum residue levels stipulated by the EU legislation on plant protection products.

The only statistically significant difference between broiler chickens fed a diet with maize NK603 and chickens fed the control maize diet occurred for fat pad weight, which was 0.034 kg and 0.037 kg (1.5% and 1.7% of body weight), respectively (Taylor et al., 2003). The fat pad weight of both NK603 and control maize were within the natural variation (0.024-0.063 kg) reported in the literature (Esteve-Garcia and Llaurado, 1997; Kidd and Kerr, 1997; Lei and Van Beek, 1997; Smith et al., 1998; Farran et al., 2000; Peak et al., 2000), and the difference was so small between treatment groups that it was not considered to be of biological relevance.

The EFSA GMO Panel also noted several published feeding studies comparing the nutritional wholesomeness of maize NK603 and appropriate non-GM control maize on Angus-continental cross steers, Holstein dairy cows, and growing-finishing pigs of two races (Fischer et al., 2002; Erickson et al., 2003; Grant et al., 2003; Ipharraguerre et al., 2003; Hyun et al., 2004). These studies show that the nutritional value of maize NK603 is equivalent to that of the control maize. A nutritional equivalence of maize NK603 to isogenic non-GM maize was also demonstrated in rats by independent investigators (Chrenková et al., 2002).

5.1.6. Post-market monitoring of GM food/feed

The risk assessment concluded that no data have emerged to indicate that maize NK603 is any less safe than its non-GM comparators. In addition, no biologically relevant agronomic and compositional changes were identified in maize NK603. Therefore, in line with the guidance document (EFSA, 2006a), the EFSA GMO Panel is of the opinion that post-market monitoring of the GM food/feed is not necessary.

5.2. Conclusion

No toxicity of orally administered CP4 EPSPS and CP4 EPSPS L214P proteins was observed in acute toxicity studies in mice. The CP4 EPSPS proteins were quickly degraded in simulated gastric and intestinal fluid without formation of stable peptide fragments. Bioinformatics studies demonstrated that the CP4 EPSPS proteins show no homology to known toxic and allergenic proteins. A comparative analysis of compositional, agronomic, and phenotypic characteristics showed that maize NK603 is equivalent to conventional non-GM maize varieties except for the introduced trait. There were no indications of adverse effects in a 90-day toxicity study in rats fed diets with up to 33% grain from maize NK603. A 42-day nutritional feeding study on broiler chickens showed that maize NK603 is as wholesome as the genetically closely related non-GM control maize and commercial maize varieties included in the study. The nutritional equivalence of maize NK603 to commercial maize varieties was confirmed in feeding studies on Angus-continental cross steers, Holstein dairy cows, growing-finishing pigs of two races, and rats. The EFSA GMO Panel is of the

opinion that maize NK603 is as safe as conventional maize, and considers that no additional animal safety or nutritional wholesomeness studies are needed.

6. Environmental risk assessment and monitoring

The scope of the applications (EFSA-GMO-NL-2005-22 and EFSA-GMO-RX-NK603) is for cultivation, food and feed uses and import and processing of maize NK603. Considering the intended uses of maize NK603 including cultivation, the environmental risk assessment is concerned with potential direct and indirect effects of the cultivation and the spread of the GM plant into non-cultivated environments, as well as indirect exposure through manure and faeces from the gastrointestinal tracts, mainly of animals fed maize NK603.

The use of glyphosate-based herbicides in the management of maize NK603 is relevant and considered in section 6.1.7. However, the regulation and risk assessment of the active substance glyphosate are within the scope of Directive 91/414/EEC concerning the placing of plant protection products on the market (EC, 1991).

The environmental risk assessment was evaluated by the Spanish Competent Authority (CA) and its scientific advisory committee (the Spanish Biosafety Commission). The opinion of the Spanish Competent Authority and its Biosafety Commission is provided in Annex H of the overall opinion[5].

In its opinion, the Spanish Competent Authority and its Biosafety Commission (see Annex H) considered the following issues: (1) persistence and invasiveness, selective advantage or disadvantage; (2) potential for gene transfer; (3) genetic and phenotypic stability; (4) interactions between the GM plant and target organisms; (5) potential interaction of the GM plant with non-target organisms; (6) potential impacts of the specific cultivation, management and harvesting techniques; and (7) effects on biogeochemical processes. According to the Spanish Competent Authority and its Biosafety Commission, "*the direct effects of the GM plants have been considered negligible*". "*Nevertheless, regarding indirect effects for the use of this herbicide tolerant crop and the impacts of the specific cultivation, management and harvesting technique, the use of herbicide tolerant GM plant could have effects on i) non-target organisms, ii) weed shifts and development of herbicide resistance to glyphosate, iii) microbial biodiversity*".

6.1. Evaluation of relevant scientific data

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

Maize is highly domesticated and not generally able to survive in the environment without appropriate cultivation practices. The survival of maize is limited by a combination of low competitiveness, absence of a dormancy phase, and susceptibility to plant pathogens, herbivory and cold climate conditions. 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

[5] http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2005-249
http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2005-075

(Gruber et al., 2008; Palaudelmàs et al., 2009). Despite cultivation for centuries, maize plants do not occur outside cultivated or disturbed land in Europe.

The applicant conducted field trials at several locations in France and Germany in 2000, 2001 and 2002. Information on 21 phenotypic characteristics was provided to assess the agronomic performance of maize NK603 in comparison with non-GM maize. No biologically meaningful differences were observed (see section 4.1.3). These field trial data did not show a change in invasiveness, weediness, or fitness of maize NK603, except when glyphosate-based herbicides are applied. In addition to the data presented by the applicant, the EFSA GMO Panel is not aware of scientific reports of increased spread and establishment of maize NK603 and any change in survival capacity, including over-wintering (Bagavathiannan and Van Acker, 2008; Gruber et al., 2008; Mallory-Smith and Zapiola, 2008).

Tolerance to glyphosate only provides an agronomic advantage in cultivation where and when glyphosate-based herbicides are applied. However, survival of maize outside of cultivation in Europe is mainly limited by a combination of low competitiveness, absence of a dormancy phase, and susceptibility to plant pathogens and cold climate conditions. Since these general characteristics of maize NK603 are unchanged, herbicide tolerance is not likely to provide a selective advantage outside of cultivation in Europe. Volunteers occurring in cultivated areas will be resistant to glyphosate, but they are normally controlled with a range of other herbicides and/or by cultivation techniques (Beckie et al., 2006). Therefore, it is considered very unlikely that volunteers of maize NK603 or its progeny will differ from conventional maize varieties in their ability to survive until subsequent seasons or to establish feral populations under European environmental conditions.

Since maize NK603 has no altered survival, multiplication or dissemination characteristics except when glyphosate-based herbicides are applied, the EFSA GMO Panel is of the opinion that the likelihood of unintended environmental effects due to the establishment and survival of maize NK603 will be no different to that of conventional maize varieties. This conclusion is in line with the evaluation carried out by the Spanish Competent Authority and its Biosafety Commission on maize NK603.

6.1.2. Gene transfer

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

6.1.2.1. Plant to bacteria gene transfer

Based on current scientific knowledge (EFSA, 2004, 2007; Keese, 2008), horizontal gene transfer from GM plants to microorganisms under natural conditions is considered extremely unlikely. Since transgenic DNA is a component of many food and feed products derived from maize NK603, microorganisms in the digestive tract of humans and animals (domesticated animals and other animals feeding on fresh and decaying GM plant material) may be exposed to transgenic DNA. Moreover, exposure of microorganisms to transgenic DNA takes place in the environment during the natural decay of plant material remaining in agricultural areas after harvest.

The plant expression plasmid vector contains two adjacent plant gene expression cassettes each containing a single copy of the *ctp*2-CP4 *epsps* gene. In the first *ctp*2-*CP4 epsps* cassette, the coding sequence is regulated by the actin rice promoter and a rice intron sequence introduced upstream of the *ctp* sequence. Expression of the second *ctp*2-CP4 *epsps* cassette is regulated by an enhanced 35S CaMV promoter and a maize intron derived from a gene encoding a heat shock protein. Genes under control of prokaryotic regulatory elements conferring related traits, as expressed in the GM plants, occur in certain microorganisms in natural environments.

Taking into account the origin and nature of the *ctp*2-CP4 *epsps* gene and the lack of selective pressure in the intestinal tract and/or the environment, the likelihood that horizontal gene transfer would result in increased fitness on microorganisms or other selective advantages is very limited. For this reason, the EFSA GMO Panel concludes that it is very unlikely that *ctp*2-CP4 *epsps* genes from maize NK603 would become transferred and established in the genome of microorganisms in the environment or in the human and animal digestive tract. In the unlikely event that such horizontal gene transfer would take place, no adverse effects on human and animal health or the environment are expected as no new traits would be introduced or expressed into microbial communities.

6.1.2.2. Plant to plant gene transfer

Maize is a cross-pollinated 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 90μm) and heavy (0.25μg) (Raynor et al., 1972, Digiovanni 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). Although vertical wind movements or gusts during pollen shedding can lift pollen up high in the atmosphere and distribute it over significant distances, concentrations of viable pollen considerably decrease with height (Aylor et al., 2003) and distance (Jarosz et al., 2005) from the source. Hence, low levels of cross-pollination can occur over longer distances under suitable climatic conditions (Bannert and Stamp, 2007; Delage et al., 2007), but most cross-pollination events occur within 50m 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; Sanvido et al., 2008).

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 hybrids and backcross progeny as well as exposure to non-target organisms.

Theoretically, seeds originating from the cross-pollination of certain cross-compatible wild/weedy relatives can mediate the potential spread and establishment of hybrids and backcross progeny (Wilkinson et al., 2003; Morales and Traveset, 2008; Devos et al., 2009a). However, in the European Union (EU), there are no cross-compatible wild/weedy relatives with which maize can hybridise and form backcross progeny (Eastham and Sweet, 2002). The only recipients of cross-pollinated transgenes from 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). Thus cross-pollination in maize is not considered an environmental risk, but is an agricultural management and coexistence issue and is not within the remit of the EFSA GMO Panel.

Even though accidental seed dispersal of maize is occurring during its cultivation in many countries, the seed-mediated establishment of maize NK603 and its survival outside of cultivation has not been reported in spite of extensive cultivation and accidental seed dispersal. Since maize plants have lost their ability to release seeds from the cob, most seed dispersal is due to harvesting and post-harvest activities of farmers. However, the survival of maize is limited by a combination of low competitiveness, absence of a dormancy phase, and susceptibility to plant pathogens, herbivory and cold climate conditions.

In conclusion, since maize NK603 has no altered survival, multiplication or dissemination characteristics except in the presence of glyphosate, the EFSA GMO Panel is of the opinion that the likelihood of unintended environmental effects as a consequence of spread of genes from maize NK603 is considered to be extremely low. This conclusion is in line with the evaluation carried out by the Spanish Competent Authority and its Biosafety Commission on maize NK603.

6.1.3. Interactions between the GM plant and target organisms

This point was not considered an issue by Member States and the EFSA GMO Panel as maize NK603 was not developed to interact with any specific target organisms. Maize NK603 was developed to allow direct application of glyphosate-based herbicides during cultivation. These herbicides have a broad spectrum of target plant species, and potential impacts of the specific cultivation are considered in section 6.1.7.

6.1.4. Interactions between the GM plant and non-target organisms

The applicant reported and supplied supplementary information on laboratory and field studies performed inside and outside the EU. These studies showed no effects of the CP4 EPSPS pure protein or tissues from maize NK603 on different types of non-target organisms (vertebrates, invertebrates and microorganisms). The organisms tested in the supplementary laboratory and field study provided by the applicant at the request of the EFSA GMO Panel, included bees (larvae and adults) and coccinellid beetles, respectively. The applicant also reported the effects of other glyphosate tolerant crops (GM soybean) on pest and beneficial organisms. Some of the studies suggested that the abundance of some beneficial organisms decreased in glyphosate tolerant crop fields compared to conventional crop fields (Jasinski et al., 2003). However, these reductions do not seem to be directly associated with the expression of CP4 EPSPS protein in herbicide tolerant crops, but are likely to be a consequence of the changes in weed populations caused by different weed management regimes.

The studies carried out by the applicant indicate that the protein CP4 EPSPS and maize NK603 have no direct effects on non-target invertebrates. Maize NK603 and other glyphosate tolerant crops have been extensively cultivated in North and South America and elsewhere for several years. The EFSA GMO Panel is not aware of any reports of direct effects on non-target organisms due to this trait and makes note of recent publications showing that there is

no evidence that GM glyphosate tolerant crops have a direct effect on biological diversity or species abundance within the planted fields (Firbank et al., 2003a; Owen, 2008). However, indirect effects on beneficial arthropods and soil microorganisms (see section 6.1.7) may occur depending on the weed management regime applied to the crop, and there are several reports of these potential indirect effects (reviewed by Cerdeira and Duke, 2006).

The EFSA GMO Panel concludes that it is unlikely that maize NK603 will have direct adverse effects on non-target organisms and considers the likelihood of adverse impacts of the specific cultivation of maize NK603 in section 6.1.7. This conclusion on the absence of direct effects of maize NK603 to non-target organisms is in line with that of the Spanish Competent Authority and its Biosafety Commission on maize NK603.

6.1.5. Effects on human and animal health

No adverse effects on human health are indicated by the molecular analysis and compositional and toxicological data supplied (see section 3 to 5).

The applicant presented several nutritional studies in which a variety of mammalian and avian species were fed with maize NK603 or diet containing NK603. The studies included a 42-day broiler chicken study and a 90-day toxicity rat study. No adverse effects on the health of these and related animals are indicated (see section 5).

6.1.6. Effects on biogeochemical processes and interaction with the abiotic environment

No direct effects of maize NK603 have been reported by the applicant on the abiotic environment and on biogeochemical processes**.** In addition, the EFSA GMO Panel is not aware of any reports of effects on the abiotic environment and on biogeochemical processes due to this trait, but is aware of several reports of the effects of the associated herbicide management, as reviewed by (Cerdeira and Duke, 2006). Glyphosate can have effects on soil microbial communities, mycorrhizal fungi and rhizobium species important in plant nutrient cycling (Zablotowicz and Reddy, 2004, 2007; Means et al., 2007; Powell et al., 2009a).

The EFSA GMO Panel concludes that it is unlikely that maize NK603 will have direct adverse effects on the abiotic environment and on biogeochemical processes. The likelihood of adverse impacts of the specific use of glyphosate is considered in section 6.1.7. This conclusion on the absence of direct effect of maize NK603 on the abiotic environment and on biochemical processes is in line with the evaluation carried out by the Spanish Competent Authority and its Biosafety Commission on maize NK603.

6.1.7. Impacts of the specific cultivation, management and harvesting techniques

6.1.7.1. Herbicide regimes in maize cropping systems

Herbicide regimes in non-genetically modified herbicide tolerant (GMHT) maize: The sensitivity of maize to early weed competition is well-understood and the need for efficient weed control early in the life of maize currently requires residual herbicide use. In principle, three different herbicide-based weed management approaches are possible in non-GMHT maize for the control of annual and perennial grass and broadleaf weeds: (1) pre-emergence of the crop; (2) early post-emergence, ideally in the 2-4 leaf stage of maize; or (3) sequentially, where a

combination of herbicides with soil (residual) activity is applied pre-emergence followed by a mixture of post-emergence herbicides with foliar activity. If these early season herbicides fail to control the weeds, an additional herbicide treatment may be applied at a later growth stage of the maize. However, these herbicides do not always provide consistent season-long control of late-emerging and/or biennial and perennial weeds (Beckie et al., 2006).

Glyphosate is a broad-spectrum contact systemic herbicide used for the control of most annual and many perennial weeds (Duke and Powles, 2008), but with no soil acting or residual properties. In the EU, glyphosate is currently used in conventional cropping and can be used pre-emergence of the crop and for cleaning of seedbed of emerged weeds. In addition, in some situations, glyphosate can be applied in an emerged crop as a band application between crop rows (Monsanto, 2007a).

Herbicide regimes in GMHT maize: glyphosate is applied post-crop emergence to established weeds providing high levels of weed control and little or no injury to the GMHT crop. Theoretically, the biotechnology-based weed management strategy enables delaying the post-emergence application of a broad-spectrum herbicide after full weed emergence, compared to non-GMHT maize (Gianessi, 2005; Cerdeira and Duke, 2006). Because the efficacy of glyphosate at controlling weeds is less dependent on weed size, glyphosate can be used up to a later growth stage for weeds. Therefore, the biotechnology-based weed management strategy offers a greater flexibility in timing of weed management. However, the control of larger and perennial weeds might require higher application rates (Monsanto, 2007a).

In the absence of pre-emergence herbicides, a sequential application of glyphosate might be needed to control weeds adequately all season. Experimental research has shown that a single post-emergence application of glyphosate alone at the recommended application rates might be inadequate (Gianessi et al., 2002; Gower et al., 2003; Parker et al., 2006). When the first single treatment is applied too early, any late-emerging weeds that remained ungerminated at the time of spraying are unaffected. These weeds can reduce crop yield by competing for resources and might set seed that replenishes the seed bank, or survive vegetatively until the following season, increasing weed pressure in subsequent years (e.g., Myers et al., 2005). Recommended strategies to avoid weed reinfestation involve the use of two post-emergence applications of glyphosate (Gower et al., 2003). In this respect, Monsanto proposes using glyphosate at dose rates ranging between 1440 and 2160 g/ha ai (active ingredient) in two applications in France (Monsanto, 2007a) and dose rates ranging between 1800 and 2160 g/ha ai in two applications in Spain (Monsanto, 2007b). In a field trial with maize NK603 in Czech Republic, optimum herbicide efficacy was provided by a split application of glyphosate (1080 + 1080 g/ha ai) (Soukup et al., 2008).

Delaying the first single treatment with glyphosate can lead to yield reductions due to an extended period of early weed competition (Gower et al., 2002, 2003; Champion et al., 2003; Cox et al., 2006). To limit early-season competition and ultimately maize yield losses, and to eliminate the need for a second post-herbicide application, the use of pre-emergence residual conventional herbicides followed by one delayed post-emergence glyphosate spray has been suggested (Thomas et al., 2004; Parker et al., 2006). In this situation, the application rates of glyphosate proposed by Monsanto are within the range of 720 to 1440 g/ha ai (Monsanto, 2007a).

In regions where early post-emergence herbicides are predominantly used, a single application of glyphosate in mixtures with other post-emergence herbicides with residual

activity is considered effective for glyphosate tolerant maize (e.g., Gianessi, 2008; Soukup et al., 2008). This will eliminate early-season weed competition and will control the weeds that are not exposed to glyphosate (Johnson et al., 2000; Thomas et al., 2004; Dill, 2005; Tharp et al., 2004; Grichar and Minton, 2006; Parker et al., 2006; Young, 2006; Zuver et al., 2006). Based on field studies conducted at 35 sites throughout the north-central US, Gower et al. (2003) concluded that the optimum timing for the 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 fourth leaf stage. In that case, the proposed glyphosate application rates are within the range of 720 to 1080 g/ha ai (Monsanto, 2007a) and dose rates of 1080 g/ha ai when mixed with another herbicide with residual activity (Monsanto, 2007b).

Examples of approved dose recommendations are 3 l/ha of glyphosate herbicide (360 g/l ai) on Roundup Ready maize in Germany[6] and a maximum of 6 l/ha of glyphosate herbicide (360 g/l ai) on Roundup Ready maize in Czech Republic[7].

In its application under Regulation (EC) No 1829/2003, Monsanto provided for information some draft recommendations on the application rates of glyphosate on maize NK603. In the Label Proposal for Roundup PRO2 submitted by Monsanto in France (Monsanto, 2007a), the applicant proposes the following application patterns and rates for the use of glyphosate on maize NK603: (1) a sequential application pre- and post-emergence at rates ranging from 720 to 1440 g/ha ai; (2) two applications post-emergence at rates ranging between 720 and 1080 g/ha ai each. A single post-emergence application of a mixture of Roundup with a residual herbicide is also being considered. The maximum annual usage dose of glyphosate is set at 2880 g/ha ai.

In the Technology Guide developed by Monsanto for the use of Roundup Ready in Spain (Monsanto, 2007b), the applicant proposes the following application patterns and rates for the use of glyphosate on maize NK603: (1) a sequential application pre-emergence with a selective herbicide with residual activity and post-emergence with Roundup Ready at rates of 1080 g/ha ai; (2) two applications post-emergence at rates ranging between from 900 and 1080 g/ha ai each; (3) single application post-emergence of a mixture (Roundup Ready + residual activity) at rates around 1080 g/ha ai.

These proposed applications are currently being reviewed by the applicant in relation to the formulation registration approval according to Annex III of Directive 91/414/EEC, but are also indicative of the range of application rates, mixtures and systems that might be applied in future.

The applicant states in its environmental risk assessment that the agronomic practices currently used to grow maize in the EU remain applicable for the cultivation of maize NK603. The applicant proposes that "*by the time of commercialisation of NK603 in the E.U., Monsanto will develop a Technology Use Guide for the European NK603 markets. This document is intended to provide more information to the farmer on Monsanto's commitment to stewardship and more detailed weed control recommendations in NK603 specific to each region. Monsanto's weed management recommendations in glyphosate-tolerant crop are based on local needs, according to crop rotation, weed species, climate and tillage regime,*

[6] https://portal.bvl.bund/psm/jsp/ListeAnwendg.jsp?ts=1242142900401
[7] http://portqal .srs.cz/portqaldoc/pripavky_na_ochranu_rostlin/informace_pro_zemedelce/registrace/vestni

using Good Agricultural Practices (GAP) as a basis. Therefore, glyphosate will not be the only weed control tool recommended in NK603".

The EFSA GMO Panel concludes that a considerable diversity of weed management regimes is likely to be used in the different agricultural regions across the EU where maize NK603 might be cultivated. EU countries show considerable variation in herbicide use in maize, weed species (including crop volunteers), meteorological and agro-environmental conditions, farming systems (including weed resistance management, rotation systems), economics, and in farmers' behaviour. Moreover, herbicide regimes are dependent on maize crop type and on weed species and biology as not all weeds are equally susceptible to glyphosate (e.g., Norsworthy et al., 2001; Soukup et al., 2008). This would mean that the locally adopted herbicide regimes and cultivation management (including conservation tillage) for GMHT maize will take into account all these factors. Therefore, it is anticipated that herbicide regimes containing glyphosate will represent different numbers of applications (single vs. sequential), doses, timing of application and the use of residual herbicides in association with glyphosate.

6.1.7.2. Interplay between Directive 2001/18/EC and Directive 91/414/EEC

Both Directives 2001/18/EC and 91/414/EEC are relevant for the risk assessment of GMHT crops (EFSA, 2008; EC, 2008). The registration and use of herbicide active ingredients in formulations in the EU is an issue for Directive 91/414/EEC as operated by individual Member States. Where herbicides are used as integral parts of the biotechnology-based weed management strategy, an environmental risk assessment must also consider their potential impact on biodiversity[8] under Directive 2001/18/EC. In the current legislation governing pesticide registration in Europe (EC, 1991), 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. For example, Kleter et al. (2007) have shown that some of the herbicides that are used on GMHT crops (e.g., glyphosate) have improved Environmental Index Quotients (EIQ) compared with comparable conventional herbicides. However, EIQs are calculated based on residual, persistence and ecotoxicity characteristics and do not relate to the efficacy and hence the biodiversity impact of herbicides. Indeed, the environmental risk assessment under Directive 91/414/EEC does not include studies of impacts on biodiversity within crops and changes in agro-ecosystems, which are required 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 (Chassy et al., 2003). It has long been recognized 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; Robinson et al., 2002). Concern has been expressed that GMHT crops, through the in-crop use of very effective broad-spectrum herbicides, will further deplete biodiversity in farmland.

[8] The term 'biodiversity' is not defined in Directive 2001/18/EC. EFSA GMO Panel regards biodiversity (= biological diversity) as the variability among living organisms from all sources, including terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this variability may include diversity within species, between species and of ecosystems

6.1.7.3. Factors affecting impacts in Europe

Extensive research has shown that impacts on the environment depend upon a wide range of baselines and on agronomic and environmental factors, which vary from region to region and from season to season. For example, Firbank et al. (2003b) commented following the UK Farm Scale Evaluations that major sources of variation in potential impacts would arise from probable future changes in agricultural practice such as herbicide regimes, tillage systems and crop rotations and from possible long-term interactions between weed and invertebrate populations. Most importantly, they stressed that the impact on biodiversity depends greatly upon the management of crops, rotations, and upon the provision of forage and habitat resources across the entire farmed landscape. Included in crop management is the dose being applied and the time and frequency of applications of the specific non-selective and other herbicides (Champion et al., 2003). Timing of application is particularly important, since with broad-spectrum herbicides sprays are 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.

All of the factors above will vary from region to region, from Member State to Member State, and from season to season. They depend not only on the nature of the particular receiving environment, but on weed pressure, soil type and climatic conditions. For these reasons, the EFSA GMO Panel recognises that there are considerable challenges to the drawing of meaningful conclusions on the environmental consequences of the use of herbicides that includes consideration of every issue involved, 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 focus of the environmental risk assessment should be on regions where the GM crop will be cultivated. Therefore, the EFSA GMO Panel considers that the future zonal system for mutual recognition foreseen in the proposed Regulation, which is expected to replace Directive 91/414/EEC, is likely to be too coarse to be used for environmental risk assessment of GM plants, since the environmental variation between arable ecosystems within zones (and indeed within many Member States) is often as large as that between zones. Two additional factors hamper the accuracy of estimates of impacts attempted at the European or zonal scale. First, individual Member States operate different regulations concerning certain aspects of conventional herbicide management applied to potential non-GM comparator crops, so there are difficulties in the quantification or establishment of detailed baselines in such dynamic situations for comparative analysis (Champion et al., 2003; Firbank et al., 2003a; Heard et al., 2005). Second, each Member State will have different baselines for the impact of current farming practices on the environment. These will influence Member State's policies on what are termed variously: environmental stewardship for farmland, biodiversity action plans, integrated pest management, good farming practice, etc.

6.1.7.4. Environmental impacts of herbicide regimes used in GMHT cropping systems

Maize NK603 is tolerant to glyphosate-based herbicides meaning that these herbicides can be directly applied to the growing crop to give effective control of weeds (Beckie et al., 2006;

Soukup et al., 2008). There is extensive literature on the range of effects of the use of glyphosate and its associated management in glyphosate tolerant crops (Cerdeira and Duke, 2006). Few studies have focussed specifically on the impacts of glyphosate associated with GMHT maize in Europe, though there is information on GMHT maize from Albajes et al. (2007, 2009) and Soukup et al. (2008). In addition, projects such as the project on botanical and rotational implications of GM herbicide tolerance in winter oilseed rape and sugar beet (BRIGHT) (Sweet et al., 2004) and the Farm Scale Evaluations (FSE) (Firbank et al., 2003a,b) in the United Kingdom and the NERI study in Denmark (e.g., Strandberg and Pedersen, 2002) have studied GMHT sugar beet and fodder beet treated with glyphosate. Also, there is information from the FSE on GMHT forage maize treated with glufosinate. Additionally there are some other studies of herbicide tolerant crops in European countries that have compared conventional production systems with GMHT systems (Madsen and Jensen, 1995; Bückmann et al., 2000; Coyette et al., 2002). Adverse effects on biodiversity of the management of glyphosate tolerant crops have been reported in several experimental studies in Europe (Brooks et al., 2003; Hawes et al., 2003; Heard et al., 2003a,b; Lutman et al., 2008; Squire et al., 2009). Although cumulative effects on biodiversity due to the continuous cultivation of a GMHT crop have been predicted (Heard et al., 2006), such effects have not been confirmed by field data. By contrast, for GMHT maize treated with glufosinate the FSE reported a generally greater abundance of biodiversity than for conventionally treated maize. Perry et al. (2004) reported that these conclusions for maize would likely be affected in degree but not in direction by the withdrawal of atrazine from conventional herbicide management. Following the FSE, the advice from the UK Advisory Committee for Releases to the Environment (ACRE) was that GMHT maize tested in FSE could be cultivated in the UK using the regimes used in the FSE, because this would not result in adverse effects, as defined and assessed by criteria specified in Directive 2001/18/EC. This advice was adopted by the Department of Environment and Rural Affairs in the UK (DEFRA, 2005). In its previous scientific opinions on maize Bt11 and 1507, the EFSA GMO Panel considered that the use of glufosinate ammonium in the cultivation of these two maize events is not likely to give an increased impact on biodiversity in most situations (EFSA, 2005a,b).

Clearly, for reasons of feasibility, practicability and cost, studies of the FSE extent could not be carried out 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 NK603 (e.g., Qi et al. 2008). The above studies have confirmed that effects on weed populations, and hence biodiversity, are very dependant on the management of the herbicides in GMHT and conventional crop production systems and on the herbicides used in both systems. In some circumstances, such as with high dosage or repeated applications, the use of glyphosate with maize NK603 will more than likely result in reductions in botanical diversity in maize fields which in turn might adversely affect food chains and webs. For this reason, the EFSA GMO Panel agrees with the statement from the applicant that '*the weed management regime, among other crop management features, may have adverse effects on non-target organisms*'.

Environmental risk assessment must recognise that farming systems are highly dynamic, and that the introduction of widespread broad-spectrum herbicide systems may lead to substantial changes in management and biodiversity. On the negative side, there is evidence from cultivation of GMHT crops in US that continuous and repeated application of glyphosate is causing changes in weed flora and development of more resistant or tolerant weeds (Fernandez-Cornejo et al., 2002). Weed shifts have also been discussed by Marshall et al.

(2001) and noted by Owen (2008). Powles (2008) has observed the development of resistance in some weed species and its effect in inducing modification of farmers' weed management through intensification of herbicide usage and subsequent adverse environmental effects. On the positive side, there is a range of beneficial effects (e.g., increases in collembolans, reduction of soil erosion, reduction in virus infection), due to the retention of weed coverage of the soil surface, during the early growth of the crop (Brookes et al., 2003; Dewar et al., 2003; May et al, 2005). In addition, the use of a broad-spectrum herbicide to control both monocotyledon 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). Changes to rotations themselves are also likely, and may have considerable effects since in BRIGHT (Lutman et al., 2008) and in the FSE (Firbank et al., 2003b) differences between the crops were comparable to those between treatments. Furthermore, the use of glyphosate-based herbicides allows greater adoption of minimal or conservation tillage (Locke et al., 2008; Givens et al., 2009). In addition, since glyphosate is broad-spectrum but has a relatively benign EIQ value it may replace alternative selective herbicides used on conventional crops which have a poorer ecotoxicological or environmental profile (Devos et al., 2008; Kleter et al., 2008). For fodder beet treated with glyphosate, Strandberg and Pedersen (2002) noted 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. They concluded that to predict the long-term consequences of GMHT systems on arable land biodiversity it would be necessary to study the effects over several seasons in relevant crop rotations. Heard et al. (2005) believed that growers might learn to tolerate higher weed densities at certain periods of the growing cycle, provided that the weeds do not cause economic loss, whilst noting that high weed densities at critical periods can seriously depress crop yields. Heard et al. (2005) predicted future changes in the timing of herbicide applications as had already occurred in the US, where uptake of GMHT crops was driven by the perceived profitability of cropping. There, glyphosate was applied earlier, and glufosinate later in the season than when GMHT crops were first introduced. The spatial nature of effects may also be important. Thus, 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. For example, farmland birds that forage extensively on weed seeds in winter, aggregating in direct response to their abundance, may be particularly affected. The complex nature of all these dynamic effects will of course be further modulated by market forces and agricultural economics.

Glyphosate can also have effects on soil microbial communities, mycorrhizal fungi and rhizobial populations important in plant nutrient cycling (Zablotowicz and Reddy, 2004, 2007; Means et al., 2007; Powell et al., 2009a). Zablotowicz and Reddy (2004) reported that glyphosate was toxic to certain *Rhizobia* involved in root nodulation and nitrogen fixation in comparison with herbicides used on conventional soybean. The consequences of this could be that glyphosate applications will reduce rhizobial populations, at least temporarily, thus reducing microbial functions and contributions to field ecosystems - principally in relation to fixing nitrogen. This could lead to increases in synthetic nitrogen application with consequences for the environment, especially water run-off etc. Powell et al. (2009b) observed that glyphosate use significantly reduces maize litter decomposition although the glyphosate effect is dependant on the location of litter placement.

From the above, the EFSA GMO Panel concludes that whilst it may be easy to list the environmental advantages and disadvantages to the adoption of GMHT systems, it is by no means simple to weigh these in the balance. Experimentation with GMHT systems may be very expensive, particularly because of the need for large-scale plots with sufficient replication (Perry et al., 2003) and of the need to sample a wide range of biodiversity (Firbank et al., 2003a) over a sufficiently long period (Lutman et al., 2008). When, for whatever reason, experimentation is deemed infeasible, modelling may be attempted, particularly to assess regional-scale and long-term effects 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 FSE. In an initial assessment, Heard et al. (2003a,b) used long-term data from the decline in UK weed seedbanks and compounded this with the reduction in 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 seedbanks of 7% per annum for a 5-course cereal rotation with a break crop grown every 5 years. By contrast, they believed that it was quite possible that, under rotations including glufosinate 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 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 rape crops in the UK be largely replaced by GMHT varieties and managed as in the FSE, 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-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.

Studies have shown that appropriate management of glyphosate can mitigate some of these potential environmental effects. Mitigation measures include protecting adjacent land from herbicide effects and the approval for the application of glyphosate (360g/l ai) on Roundup Ready maize in Germany[9] includes recommendations for separation distances of 20 m from certain sensitive areas and measures for the protection of water courses. Other measures include reducing the proportion of the field or crop that is glyphosate treated in order to maintain levels of biodiversity. For example, Dewar et al. (2003); May et al. (2005) and Pidgeon et al. (2007) described mitigation measures that could be applied to glyphosate tolerant sugar beet and similar measures could be applied to maize NK603.

In conclusion, the EFSA GMO Panel is of the opinion that potential impacts on the specific cultivation, management and harvesting techniques of maize NK603 are indirect effects entirely associated with the use of the complimentary herbicide regimes and that if maize

[9] https://portal.bvl.bund/psm/jsp/ListeAnwendg.jsp?ts=1242142900401

NK603 was grown in a similar manner to conventional maize, it would not cause additional adverse environmental impacts. These conclusions are in line with the conclusions of the Spanish Competent Authority and its Biosafety Commission. These potential adverse environmental effects comprise (1) the evolution of less desirable weed assemblages leading to a reduction in farmland biodiversity; (2) the evolution of weed resistance and (3) the effects on soil microbial communities. The magnitude of these potential adverse environmental effects will depend on the specific herbicide management applied at the farm level.

6.1.8. Conclusions

Since the scope of the current applications includes cultivation, the environmental risk assessment considered the environmental impact of full-scale commercialisation.

The EFSA GMO Panel considers that maize NK603 has no altered survival, multiplication or dissemination characteristics and interacts with other organisms as conventional maize. The likelihood of unintended environmental effects due to the establishment and spread of maize NK603 will be no different from that of traditionally bred maize.

The EFSA GMO Panel is of the opinion that potential adverse environmental effects of the cultivation of maize NK603 are indirect effects entirely associated with the use of the complimentary herbicide regimes. These potential adverse environmental effects comprise (1) the evolution of less desirable weed assemblages leading to reductions in farmland biodiversity; (2) the evolution of weed resistance; and (3) effects on soil microbial communities. The magnitude of these potential adverse environmental effects will depend on the specific herbicide management applied at the farm level.

Thus the EFSA GMO Panel concludes that maize NK603 plants are unlikely to cause any direct adverse effects, but that potential adverse environmental effects of the cultivation of maize NK603 associated with the use of the complimentary glyphosate herbicide have been identified. This conclusion is in line with the conclusions of the Spanish Competent Authority and its Biosafety Commission.

6.2. Post-market environmental monitoring

In its environmental risk assessment report, the Spanish Competent Authority and its Biosafety Commission (see Annex H) identify the "*need to consider deeper studies on i) the potential indirect effects on non-target organisms due to the weed management, ii) the development of weed resistance to glyphosate and iii) the evolution of the flora associated to management of the cultivation of maize NK603 and their potential impact on biodiversity*". The Spanish Competent Authority and its Biosafety Commission consider that "*these potential effects may need to be considered under a post market case-specific-monitoring under different European conditions for maize NK603 varieties and additional studies may be required upon agreement with the National Competent Authority*". The Spanish Competent Authority and its Biosafety Commission are of the opinion that the general surveillance proposal and the use of questionnaires are appropriate. However, the Spanish Competent Authority and its Biosafety Commission note that "*the questions in the questionnaire mainly refer to the period in which the GM crop is in the field and does not take sufficiently into*

account the possible development of resistant herbicide tolerant weeds or their impact on biodiversity at a later stage".

6.2.1. General aspects of monitoring

The objectives of a post-market environmental monitoring (PMEM) plan according to Annex VII of Directive 2001/18/EC are (1) to confirm that any assumption regarding the occurrence and impact of potential adverse effects of the GMO, or its use, in the environmental risk assessment are correct; and (2) to identify the occurrence of adverse effects of the GMO, or its use, on human health or the environment that were not anticipated in the environmental risk assessment.

The EFSA GMO Panel notes that it only gives its opinion on the scientific quality of the post-market environmental monitoring activities proposed by applicants, whilst the final endorsement thereof is done by risk managers.

The EFSA GMO Panel is of the opinion that the structure of the post-market environmental monitoring plan provided by the applicant complies with the requirements defined in the Directive 2001/18/EC and the EFSA GMO Panel guidance on post-market environmental monitoring (EFSA, 2006b).

6.2.2. Interplay between environmental risk assessment and monitoring

The environmental risk assessment of maize NK603 concluded that (1) maize NK603 plants are unlikely to cause any direct adverse effects; and that (2) potential adverse environmental effects of the cultivation of maize NK603 are associated with the use of the complimentary glyphosate-based herbicides. These potential adverse environmental effects comprise (1) the evolution of less desirable weed assemblages leading to reductions in farmland biodiversity; (2) the evolution of weed resistance; and (3) effects on microbial communities. The magnitude of these potential adverse environmental effects will depend on the specific herbicide management applied at the farm level. In line with the requirements of Directive 2001/18/EC (EC, 2001) and the EFSA GMO Panel interplay working document (EFSA, 2008) the EFSA GMO Panel recommends using glyphosate on maize NK603 only in herbicide regimes that have similar or reduced environmental impacts compared with conventional maize cultivation.

The EFSA GMO Panel considers that measures could be put in place under Directive 91/414/EEC to ensure compliance with regulatory requirements of the pesticide regimes operating in Member States. These could 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 NK603. The occurrence of weed resistance would be reported to each Member State on an annual basis and could also be submitted to organisations such as the European Weed Research Association who monitor weed resistance and develop international strategies for managing weed resistance.

Moreover, under Directive 91/414/EEC, effects of active substances on soil microbial communities are considered through functional tests on nitrification and soil respiration. The

specific use of glyphosate during the growing season of GMHT crops can therefore be included in such tests.

6.2.3. Case-specific monitoring

The EFSA GMO Panel agrees with the environmental risk assessment performed by the applicant that no direct adverse environmental effects of maize NK603 have been observed and are anticipated and thus case-specific monitoring of the GM plant itself is not required.

The Spanish Competent Authority and its Biosafety Commission proposed that monitoring should be conducted under Directive 2001/18/EC and recommended to "*consider deeper studies on the following potential adverse effects: the potential indirect effects on non-target organisms due to the weed management, the development of weed resistance to glyphosate and the evolution of the flora associated to management of the cultivation of NK603 maize and their potential impacts on biodiversity*".

However, the EFSA GMO Panel refers to the lessons learned from the Farm Scale Evaluations of genetically modified herbicide-tolerant crops (e.g., Firbank et al. 2003; Qi et al. 2008) (see section 6.1.1.7.d). The EFSA GMO Panel is of the opinion that an alternative option to deeper post-risk assessment studies would be the use of management and mitigation measures (e.g., Dewar et al., 2003; May et al., 2005; Pidgeon et al., 2007) to manage herbicide effects on biodiversity in conjunction with the monitoring for weed resistance evolution under Directive 91/414/EEC as proposed above. General surveillance (Directive 2001/18/EC) is used to determine unanticipated adverse environmental effects.

6.2.4. General surveillance

The objective of general surveillance is to identify unforeseen adverse effects of the GM plant or its use on human health and the environment that were not predicted in the risk assessment.

The general surveillance proposed by the applicant is based on four pillars: (1) the use of annual farm questionnaires; (2) the review of scientific information provided by existing observations networks; (3) the implementation of company stewardship programs; and (4) the follow-up of various information sources such as official websites, scientific publications and expert reports on GMOs to identify potential adverse effects associated with the intended uses of maize NK603.

The EFSA GMO Panel considers general surveillance for the environmental effects of maize NK603 cultivation to be in line with the general recommendations of its guidance on post-market environmental monitoring (EFSA, 2006b).

The EFSA GMO Panel welcomes the approach of the applicant to establish farm questionnaires as a reporting format. The questionnaires to farmers exposed to or using maize NK603 provided by the applicant are regarded as an adequate tool for addressing several aspects of general surveillance. While the EFSA GMO Panel considers the format of the questionnaires provided by the applicant as comprehensive, it proposes the following modifications:

- Reconsideration whether the alternative response "*don't know*" or similar ones should be added to the answering options to prevent false answers;

- Questions should be added on the occurrence/observation of (GM) feral plants and/or (GM) volunteers in subsequent seasons (for the consideration of persistence or selection);

- Independent from the occurrence/abundance of wildlife, an open question/answer should address "*unexpected observations*" (... "*if – please specify*" for the consideration of effects on non-target organisms);

- The questionnaire should be designed to allow for the input of general farm information (e.g., crop rotations, crop performance, crop yields) and field-specific information (e.g., data on fertilizer usage, soil fertility, pests and diseases, pesticide use and weed abundance) for each GMHT maize field that is being monitored. In addition, the questionnaire should include an advisory note explaining that separate data sets are required for each maize NK603 field to be monitored on a single farm;

- Farm questionnaires for the year(s) after the GM maize cultivation need to be adapted for the monitoring of the specific crops (maize or different) that follow the maize NK603 cultivation. It should be in a format that is statistically compatible with the questionnaires supplied for the maize NK603 growing season;

- In addition to the monitoring of GM herbicide tolerant crops for unanticipated adverse environmental effects (as part of the general surveillance activities), the applicant should describe the information which will be collected to assess whether the herbicide management strategies of Member States are being followed and the levels of weed control achieved.

The EFSA GMO Panel agrees with the proposal of the applicant to describe the generic approaches for using other existing surveillance networks. The GMO Panel reiterates its recommendations on the use of existing networks (e.g., for biodiversity monitoring) and the liaison between applicants and EU Member States as outlined in chapter 5.3 of the PMEM opinion (EFSA 2006b). 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 NK603 will be cultivated and intends to investigate their suitability for providing data on potential changes in biota. However, the EFSA GMO Panel emphasises the following requirements;

- Existing surveillance networks that monitor herbicide usage, botanical diversity on farms and weed resistance development should be specifically included in the sources of information that support general surveillance of maize NK603, in order to substantially fulfil the scientific requirements for the detection of any unforeseen environmental effects in relation to maize NK603 cultivation;

- The applicant should commit more explicitly to take into account data collected and published from existing monitoring programs;

- The applicant should be willing to work, within an appropriate time of establishment of the new monitoring commitments, with the Competent Authorities under Directive 2001/18/EC of the different Member States where the maize NK603 will be grown, to review the existing monitoring networks.

The EFSA GMO Panel also recommends some improvements of general surveillance for the following issues:

- The role and interplay of all intended actors on behalf of recording, analysis, evaluation and reporting of monitoring data should be specified and clarified transparently;

- The current monitoring plan describes the distribution and analysis of farm questionnaires by the applicant, whilst the obligation for further data collection and analysis is assigned to third parties and the Competent Authorities in the Member States. However, at this stage, no agreement on the procedure is seemingly achieved with these institutions. Moreover, it is not clear which kind of data will be collected to allow further assessment. Hence, stating to evaluate some annual reports from third parties provides no insight what is actually intended. Therefore, this aspect should be clarified by the applicant before market consent is given.

6.2.5. Reporting results of monitoring

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

6.2.6. Conclusions

The EFSA GMO Panel recommends that the use of the associated herbicide does not cause adverse environmental impacts greater than those associated with the cultivation of conventional maize in the EU. This is in line with requirements of Regulation (EC) No 1829/2003 and those of Directive 2001/18/EC. Herbicide management in maize NK603 cultivation should be designed to manage environmental impacts and monitoring should record any evolution of resistant weeds and adverse unanticipated environmental effects.

The EFSA GMO Panel is of the opinion that monitoring of herbicide management, compliance with pesticide regulatory requirements of each Member State and effects on weed resistance, is required in each Member State permitting the use of glyphosate on maize NK603. This should be done under the pesticide regimes operating in Member States in compliance with Directive 91/414/EEC as well as under general surveillance under Directive 2001/18/EC to determine unanticipated adverse environmental effects. The Spanish Competent Authority and its Biosafety Commission propose that monitoring should be conducted under Directive 2001/18/EC and recommend to "*consider deeper studies on the following potential adverse effects: the potential indirect effects on non-target organisms due to the weed management, the development of weed resistance to glyphosate and the evolution of the flora associated to management of the cultivation of NK603 maize and their potential impacts on biodiversity*". In the frame of general surveillance, the Spanish Competent Authority and its Biosafety Commission consider the use of farm questionnaires as sole monitoring means insufficient for the detection of unexpected environmental effects related to the cultivation of

maize NK603. Therefore, they have proposed that case-specific monitoring is conducted under Directive 2001/18/EC.

However, the EFSA GMO Panel is of the opinion that an alternative option would be the use of management and mitigation measures to reduce the adverse environmental effects of the herbicide, in conjunction with the monitoring for weed resistance evolution under Directive 91/414/EEC and general surveillance monitoring under Directive 2001/18/EC to determine unanticipated adverse environmental effects.

The EFSA GMO Panel notes that the general surveillance plan not only relies on farm questionnaires, but also on other sources of data input (such as stewardship programs, literature screenings). In principle, the EFSA GMO Panel agrees with the general methods and approaches of the general surveillance plan, but advises the applicant to describe in more detail how information will be collected that could be used to assess whether the intended uses of maize NK603 and its specific management are having unanticipated adverse environmental effects. The EFSA GMO Panel is content with the generic plan of the applicant to liaise with Competent Authorities in the Member States to implement an EU-wide post-market environmental monitoring plan on a national level.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Maize NK603 has been developed for tolerance to glyphosate by the introduction of a gene coding for glyphosate tolerant 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) derived from *Agrobacterium* sp. strain CP4 (CP4 EPSPS).

The EFSA GMO Panel assessed maize NK603 with reference to the intended uses and appropriate principles described in the guidance document of the EFSA GMO Panel for the risk assessment of GM plants and derived food and feed.

The EFSA GMO Panel is of the opinion that the molecular characterisation provided for the transformation event NK603 is sufficient for the safety assessment. The bioinformatic analysis of the inserted DNA and flanking regions does not raise any safety concern. The expression of the genes introduced by genetic modification has been sufficiently analysed and the stability of the genetic modification has been demonstrated over several generations. The EFSA GMO Panel considers that the molecular characterisation does not indicate any safety concern.

The Panel has considered information provided on (1) the molecular inserts within the transgenic event; (2) the chemical composition of the GM and non-GM maize; and (3) the safety of the proteins expressed (CP4 EPSPS and CP4 EPSPS L214P) and the whole maize NK603. Based on the results of compositional analysis of grain and forage material of maize NK603 collected at field trials from a representative range of environments and seasons, the EFSA GMO Panel concludes that maize NK603 is compositionally equivalent to conventional maize, except for the presence of the CP4 EPSPS proteins. In addition, field trials did not show changes in phenotypic characteristics and agronomic performance except for the introduced trait.

The CP4 EPSPS and CP4 EPSPS L214P proteins did not induce adverse effects in studies on acute oral toxicity in mice. There were no adverse effects in a 90-day feeding study on rats with maize NK603 grain. Feeding studies on broiler chickens, Angus-continental cross steers,

Holstein dairy cows, growing-finishing pigs, and rats provided evidence of nutritional equivalence of maize NK603 to conventional maize. In addition it is unlikely that the overall allergenicity of the whole plant is changed. The EFSA GMO Panel is of the opinion that maize NK603 is as safe as conventional maize. Maize NK603 and derived products are unlikely to have any adverse effect on human and animal health in the context of the intended uses.

On 25 March 2008, the Spanish Competent Authority and its Biosafety Commission provided to EFSA its opinion on the environmental risk assessment in line with Articles 6.3 (e) and 18.3 (e) of Regulation (EG) No 1829/2003. The Spanish Competent Authority and its Biosafety Commission conclude that "*according to the current state of scientific knowledge and after examining the existing information and data provided by the Monsanto Company, the Spanish Commission on Biosafety could give a favourable opinion to the commercialisation in the E.U. of maize NK603 if proposals and conditions established in the ERA report are implemented*".

The EFSA GMO Panel considers that maize NK603 has no altered survival, multiplication or dissemination characteristics and interacts with other organisms as conventional maize. The likelihood of unintended environmental effects due to the establishment and spread of maize NK603 will be no different from that of traditionally bred maize. The EFSA GMO Panel considers that the potential environmental impacts of the specific cultivation, management and harvesting techniques of maize NK603 are indirect effects entirely associated with the use of the complimentary herbicide regimes. Thus the EFSA GMO Panel concludes that maize NK603 plants are unlikely to cause any direct adverse effects, but that potential adverse environmental effects of the cultivation of maize NK603 associated with the use of the complimentary glyphosate herbicide have been identified. This conclusion is in line with the conclusions of the Spanish Competent Authority and its Biosafety Commission.

The EFSA GMO Panel is of the opinion that potential adverse environmental effects of the cultivation of maize NK603 are indirect effects entirely associated with the use of the complimentary herbicide regimes. These potential adverse environmental effects comprise (1) the evolution of less desirable weed assemblages leading to reduction in farmland biodiversity; (2) the evolution of weed resistance; and (3) the effects on microbial communities. The magnitude of these potential adverse environmental effects will depend on the specific herbicide management applied at the farm level. Studies have shown that appropriate management of glyphosate can mitigate some of these potential environmental effects (e.g., Dewar et al., 2003; May et al., 2005; Pidgeon et al., 2007; see section 6.1.7.d).

The EFSA GMO Panel recommends that the potential adverse effects of the glyphosate should be evaluated for the specific use on maize NK603 during the national registration by Member States under the pesticide Directive 91/414/EEC. In addition, the EFSA GMO Panel recommends that the occurrence of weed resistance and appropriate management strategies should be addressed as part of the registration of glyphosate under Directive 91/414/EEC. In line with its interplay working document (EFSA, 2008) and the requirements of Directive 2001/18/EC (EC, 2001), the EFSA GMO Panel also recommends using glyphosate on maize NK603 in management regimes that have similar or reduced environmental impacts compared with conventional maize cultivation.

The Spanish Competent Authority and its Biosafety Commission propose that monitoring should be conducted under Directive 2001/18/EC and recommend to "*consider deeper studies*

on the following potential adverse effects: the potential indirect effects on non-target organisms due to the weed management, the development of weed resistance to glyphosate and the evolution of the flora associated to management of the cultivation of NK603 maize and their potential impacts on biodiversity".

However, the EFSA GMO Panel is of the opinion that an alternative option would be the use of management and mitigation measures to reduce the adverse environmental effects of the herbicide, in conjunction with the monitoring for weed resistance evolution under Directive 91/414/EEC and general surveillance monitoring under Directive 2001/18/EC to determine unanticipated adverse environmental effects.

The EFSA GMO Panel agrees with the general methods and approaches of the general surveillance plan, but advises the applicant to describe in more detail how information will be collected that could be used to assess whether the intended uses of maize NK603 and its specific management are having unanticipated adverse environmental effects.

In conclusion, the EFSA GMO Panel considers that the information available for maize NK603 addresses the scientific comments raised by Member States and that maize NK603 is as safe as its conventional counterpart with respect to potential direct effects on human and animal health and the environment. However, EFSA GMO Panel concludes that potential impacts on the specific cultivation, management and harvesting techniques of maize NK603 are indirect effects entirely associated with the use of the complimentary herbicide regimes. The EFSA GMO Panel therefore recommends managing the use of glyphosate on maize NK603 only in regimes that have similar or reduced environmental impacts compared with conventional maize cultivation.

DOCUMENTATION PROVIDED TO EFSA

1. Letter from the Competent Authority of the Netherlands, dated 2 August 2005, concerning a request for placing on the market of maize NK603 in accordance with Regulation (EC) No 1829/2003.
2. Acknowledgement letter, dated 14 October 2005, from EFSA to the Competent Authority of the Netherlands.
3. Letter from EFSA to applicant, dated 12 May 2006, delivering the 'Statement of Validity' for application EFSA-GMO-NL-2005-22, maize NK603 submitted by Monsanto under Regulation (EC) No 1829/2003.
4. Letter from EFSA (Spanish CA) to applicant dated 22 September 2006, requesting additional information and stopping the clock.
5. Letter from applicant to EFSA (Spanish CA), dated 15 December 2006, providing additional information.
6. Letter from EFSA to applicant, dated 08 February 2007, requesting additional information and maintaining the clock stopped.
7. Letter from EFSA (Spanish CA) to applicant, dated 15 February 2007, requesting additional information and maintaining the clock stopped.

8. Letter from applicant to EFSA, dated 8 August 2007, providing additional information.
9. Letter from applicant to EFSA (Spanish CA), dated 10 October 2007, providing additional information.
10. Letter from EFSA (Spanish CA) to applicant, dated 19 November 2007, requesting additional information and maintaining the clock stopped.
11. Letter from applicant to EFSA (Spanish CA), dated 11 December 2007, providing additional information.
12. Letter from the Spanish Competent Authority to EFSA with the environmental risk assessment report of the Spanish Biosafety Commission.
13. Letter from EFSA to applicant, dated 28 April 2008, requesting additional information and maintaining the clock stopped.
14. Letter from applicant to EFSA, dated 1 October 2008, providing additional information.
15. Letter from EFSA to applicant, dated 7 November 2008, requesting additional information and maintaining the clock stopped.
16. Letter from applicant to EFSA, dated 15 December 2008, providing additional information.
17. Letter from EFSA to applicant, dated 16 February 2009, requesting additional information and maintaining the clock stopped.
18. Letter from applicant to EFSA, dated 25 February 2009, providing additional information.
19. Letter from EFSA to applicant, dated 06 March 2009, restarting the clock.

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