Opinion of the Scientific Panel on Genetically Modified Organisms on an application (Reference EFSA-GMO-UK-2004-01) for the placing on the market of glyphosate-tolerant and insect-resistant genetically modified maize NK603 x MON810, for food and feed uses under Regulation (EC) No 1829/2003 from Monsanto[1]

(Question No EFSA-Q-2004-086)

Opinion adopted on 13 October 2005

SUMMARY

This document provides an opinion of the Scientific Panel on Genetically Modified Organisms (GMO Panel) of the European Food Safety Authority (EFSA) on genetically modified maize NK603 x MON810 (Unique Identifier MON-ØØ6Ø3-6 x MON-ØØ81Ø-6), developed to provide protection against specific lepidopteran pests and tolerance to glyphosate.

In delivering its opinion the GMO Panel considered the application (Reference EFSA/GMO/UK/2004/01) and the specific comments submitted by the Member States as well as the notification C/GB/02/M3/3 for NK603 x MON810 maize as submitted under Directive 2001/18/EC. Further information from placing MON810 and NK603 maize on the market was taken into account where appropriate. Although an overall single risk assessment of all uses, excluding cultivation, has been made, for regulatory reasons, opinions for the application under Regulation (EC) No 1829/2003 and the notification under Directive 2001/18/EC are issued separately.

NK603 x MON810 maize was 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 Genetically Modified Plants and Derived Food and Feed'. The scientific assessment included examination of the transgenic DNA present in NK603 x MON810 maize and the nature and safety of the new proteins produced by the transgenic plants with respect to toxicology and allergenicity. Furthermore, a comparative analysis of agronomic traits and composition was undertaken and the safety of the whole food/feed was evaluated. A nutritional and an environmental assessment, including monitoring plan, were both undertaken.

The single events MON810 and NK603 have been the subjects of earlier assessments. MON810 maize has been previously evaluated and approved under Directive 90/220/EEC. NK603 maize has been previously evaluated and approved under Directive 2001/18/EC. The use of food ingredients from MON810 maize and from NK603 maize were both notified under Regulation (EC) No 258/97.

[1] For citation purposes: Opinion of the Scientific Panel on Genetically Modified Organisms on an application (Reference EFSA-GMO-UK-2004-01) for the placing on the market of glyphosate-tolerant and insect-resistant genetically modified maize NK603 x MON810, for food and feed uses under Regulation (EC) No 1829/2003 from Monsanto, *The EFSA Journal* (2005) 309, 1-22.

Molecular analysis of the individual inserts in NK603 and MON810 parents included information on the complete sequence of inserts and flanking regions. The GMO Panel is of the opinion that bioinformatic analysis of the DNA insert and flanking regions indicates no cause for concern. As traditional breeding methods were used in the production of NK603 x MON810 maize, no genetic modification was involved and thus the molecular structures of the DNA inserts in NK603 and MON810 were expected to remain unchanged in NK603 X MON810. This was indicated by the preservation of the phenotypes and was further confirmed using Southern blots which demonstrated that insert structures were indeed retained in NK603 x MON810 maize.

The mean levels of Cry1Ab and CP4 EPSPS proteins in forage and grain of NK603 x MON810 were not significantly different from MON810 and NK603 maize, which were previously considered safe and approved. There were large but similar ranges in the expression of these proteins in NK603 x MON810 and in NK603 and MON810, respectively. The GMO Panel concludes that these data do not raise safety concerns.

The Panel found no evidence of any interactions between the newly expressed proteins Cry1Ab and CP4 EPSPS and there were no indications of altered allergenic potency of NK603 x MON810 as compared to non-modified maize. In addition, a compositional comparison of NK603 x MON810 maize with non-transgenic comparators revealed no relevant differences. The GMO Panel is therefore of the opinion that this hybrid between MON810 and NK603 maize is as safe for human and animal health as conventional maize. The Panel further concludes that experimental studies have shown NK603 x MON810 maize to be nutritionally equivalent to conventional maize.

The application EFSA-GMO-UK-2004-01 concerns food and feed uses. There is therefore no requirement for scientific information on possible environmental effects associated with the cultivation of the GM maize. The GMO Panel agrees that unintended environmental effects due to the adventitious establishment and spread of NK603 x MON810 maize will not be different from that of traditionally bred maize. The GMO Panel also concludes that the amounts of Cry1Ab protein being distributed onto land in animal and food waste would be very low, minimizing the possibility for exposure of potentially sensitive non-target organisms. The monitoring plan provided by the applicant is in line with the intended uses for the NK603 x MON810 maize.

In conclusion, the GMO Panel considers that the information available for NK603 x MON810 maize addresses the outstanding questions raised by the Member States and considers it unlikely that NK603 x MON810 maize will have any adverse effect on human and animal health or the environment in the context of its proposed uses.

This scientific opinion corresponds to the risk assessment report requested under Article 6(6) of Regulation (EC) No 1829/2003 and will be part of the overall opinion as required by Regulation (EC) No 1829/2003.

Key words: MON810, NK603, NK603 x MON810, GMO, maize, *Zea mays*, food/feed safety, human health, environment, import, Regulation (EC) 258/97, Regulation (EC) 1829/2003, Directive 90/220/EEC, Directive 2001/18/EC.

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BACKGROUND

On 10 June 2004 EFSA received from the Competent Authority of United Kingdom an application (Reference EFSA-GMO-UK-2004-01) for authorisation of NK603 x MON810 maize (Unique Identifier MON-ØØ6Ø3-6 x MON-ØØ81Ø-6), submitted by Monsanto within the framework of Regulation (EC) No 1829/2003 on genetically modified food and feed (EC, 2003).

After receiving the application EFSA-GMO-UK-2004-01 and in accordance with Articles 5(2)(b) and 17(2)b of Regulation (EC) No 1829/2003, EFSA informed the Member States and the Commission and made the summary of the dossier publicly available on the EFSA website[2]. EFSA initiated a formal review of the application to check compliance with the requirements laid down in Articles 5(3) and 17(3) of Regulation (EC) No 1829/2003. On 17 June 2005 EFSA declared the application as formally valid in accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003.

EFSA made the valid application available to the Member States and the Commission and consulted nominated risk assessment bodies of the Member States, including the national Competent Authorities within the meaning of Directive 2001/18/EC (EC, 2001) following the requirements of Articles 6(4) and 18(4) of Regulation (EC) No 1829/2003, to request their opinion concerning placing the product on the market. The Member State bodies had three months after the date of receipt of the valid application (until 17 September 2005) within which to make their opinion known.

The Scientific Panel on Genetically Modified Organisms carried out a scientific assessment of the genetically modified maize NK603 x MON810 for food and feed uses, in accordance with Articles 6(6) and 18(6) of Regulation (EC) No 1829/2003, taking into consideration the opinions of the Member States. Further information from applications for placing NK603 and MON810 and the hybrid (Ref C/GB/02/M3/3 submitted under Directive 2001/18/EC) on the market under EU regulatory procedures was taken into account where appropriate. Although an overall single risk assessment of all uses, excluding cultivation, has been made, for regulatory reasons, opinions for the application under Regulation (EC) No 1829/2003 and the notification under Directive 2001/18/EC (EFSA, 2005c) are issued separately.

The single events MON810 and NK603 have been the subjects of earlier assessments. MON810 maize has been previously evaluated (SCP, 1998a) and approved under Directive 90/220/EEC by Commission Decision 98/294/EC (EC, 1998a). NK603 maize has received an EFSA opinion in favour of its placing on the market (EFSA, 2004a,b; 2003a,b) and was authorised under Directive 2001/18/EC by Commission Decision 2004/643/EC (EC, 2004b). The use of food

[2] http://www.efsa.eu.int/science/gmo/gm_ff_applications/catindex_en.html

ingredients from MON810 maize was notified in 1997 under Regulation (EC) No 258/97[3] (EC, 1997; EC, 2004a). The use of food and food ingredients from NK603 maize was authorised under Regulation (EC) No 258/97 by Commission Decision 2005/448/EC (EC, 2005).

In accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003 EFSA has, in giving its opinion to the Commission, the Member States and the applicant, endeavoured to respect a time limit of six months as from the receipt of a valid application.

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

In summary, NK603 x MON810 maize has been submitted by the applicant under Directive 2001/18/EC as well as under Regulation (EC) 1829/2003. The scope of both applications is different. Import and processing of NK603 x MON810 grains were assessed according to Directive 2001/18/EC whereas food and feed uses (e.g. grains, meal, oil) of NK603 x MON810 maize were assessed according to the procedure laid down under Regulation (EC) No 1829/2003.

TERMS OF REFERENCE

The GMO Panel was requested, in accordance with Articles 6(6) and 18(6) of Regulation (EC) No 1829/2003, to carry out a scientific assessment of the genetically modified maize NK603 x MON810 for food and feed uses.

Where applicable, any conditions or restrictions which should be imposed on the placing on the market and/or specific conditions or restrictions for use and handling, including post-market monitoring requirements based on the outcome of the risk assessment and, in the case of GMOs or food/feed containing or consisting of GMOs, conditions for the protection of particular ecosystems/environment and/or geographical areas should be indicated in accordance with Articles 6(5)(e) and 18(5)e of Regulation (EC) No 1829/2003.

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

ASSESSMENT

1. Introduction

Genetically Modified (GM) maize NK603 x MON810 is assessed with reference to its intended uses and the appropriate principles described in the guidance document of the GMO Panel for

[3] According to Article 5 of Regulation (EC) No 258/97 of the European Parliament and of the Council (EC, 1997), novel foods or novel food ingredients may follow a simplified procedure, only requiring notification from the company, when they are considered by a national food assessment body as 'substantially equivalent' to existing foods or food ingredients (as regards their composition, nutritional value, metabolism, intended use and the level of undesirable substances contained therein). Notification 'Food and food ingredients produced from maize flour, maize gluten, maize semolina, maize starch, maize glucose and maize oil derived from the progeny of maize line MON810' was considered by the UK Advisory Committee on Novel Foods and Processes (ACNFP, 1996).

the risk assessment of genetically modified plants and derived food and feed (EFSA, 2004a). The combination of separate inserts as a result of a cross between GM plants raises questions about the extent to which data on NK603 and MON810 maize can be extrapolated to assess the safety of NK603 x MON810 maize. The GMO Panel regards this on a case-by-case basis in regards if a particular detail of the individual inserts is of relevance to the hybrid.

2. Molecular characterisation

2.1 Issues raised by the Member States

Questions were raised regarding (1) the structure and stability of the inserts in NK603 x MON810 maize; (2) the differences in expression of the Cry1Ab proteins in NK603, MON810 and NK603 x MON810 and (3) the variability in expression between different areas of cultivation.

2.2. Evaluation of relevant scientific data

The GMO Panel Guidance Document (EFSA, 2004a) states that when events have been combined by the interbreeding of existing approved GM lines the need for further molecular analysis will depend, on a case-by-case basis, on the nature of the genetic modifications involved. However, there is no *a priori* or biological reason to assume that traditional interbreeding of independent approved GM events will pose any additional risk through a compromised stability of copy number and insert structure.

2.2.1. Method of hybrid production

Traditional breeding methods were used to produce NK603 × MON810 and no new genetic modification was involved. The two inserts that are present in NK603 × MON810 were derived from maize lines containing two independent events: NK603 and MON810. Each of these GM maize events was the subject of an earlier safety evaluation and separate opinions (EFSA, 2003a,b; SCP 1998a) for each of them have been published. NK603 × MON810 combines the lepidopteran protection traits from MON810 with the herbicide (glyphosate) tolerance trait from NK603.

The production of hybrid maize is a well established process in traditional maize breeding. It involves the production of separate elite inbred lines that are subsequently crossed in order to produce hybrid seed that is used in agriculture. This process allows the selection of desirable traits and the crossing of inbred lines results in heterosis and a superior agricultural performance.

2.2.2. Summary of the previous evaluation of the single events

MON810

MON810 maize was developed to produce an insecticidal activity against lepidopteran insect pests by the introduction of part of a *Bacillus thuringiensis* gene encoding the insecticidal Cry1Ab protein. Particle acceleration was used to introduce plasmid PV-ZMBK07 into maize cells and subsequent molecular characterization demonstrated that the sequences actually inserted included sufficient of the *cry1Ab* coding region to encode an insecticidal Cry1Ab protein (SCP, 1998a).

MON810 maize was the subject of an earlier safety assessment (SCP, 1998a) under Directive 90/220/EEC (EC, 1990) in which the molecular characterization of the inserted transgenic DNA and its stability were evaluated. A complete DNA sequence of the insert in maize event MON810

was determined and this confirmed its predicted structure which consists of the enhanced CaMV 35S promoter, the maize HSP70 intron and part of the *cry1Ab* coding region (sufficient to encode an insecticidal Cry1Ab protein). An apparent inconsistency in bioinformatic data for the 5' flanking DNA in MON810 was clarified as resulting from searching an updated database. In addition, a specific concern about possible secondary insertions of the nos terminator in the genome of MON810 was resolved (EFSA, 2004b, c).

NK603

NK603 maize was the subject of earlier safety assessments (EFSA, 2003a,b). In the NK603 event glyphosate tolerance was achieved by the introduction of a gene encoding glyphosate tolerant 5-enoylpyruvylshikimate-3-phosphate synthase from *Agrobacterium* sp. strain CP4 (CP4 EPSPS). The EPSPS activity is needed for the biosynthesis of aromatic amino acids in plants and in micro-organisms but the structure of the normal plant enzyme makes it commonly vulnerable to glyphosate, thereby causing the plants to be killed by elevated doses of the herbicide. Use of the CP4 EPSPS gene in the transgenic plant confers tolerance to the herbicide.

Molecular analysis showed that NK603 contains a single inserted copy of the DNA present in the construct used for the transformation. The plasmid vector contains two adjacent plant gene expression cassettes each containing a single copy of the *cp4 epsps* gene. The insert in NK603 does include some molecular rearrangements at one end of the insert and also includes a fragment of chloroplast DNA. These rearrangements and the insertion of chloroplast DNA do not lead to new traits and are not considered to pose a safety risk. In the unlikely event that a new peptide or protein is produced as a consequence of the insertion event, bioinformatics analysis showed that these would have no homology to known toxins or allergens.

2.2.3. Transgenic constructs in the hybrid

The molecular structures of the DNA inserts present in NK603 x MON810 maize were investigated using Southern analyses. These were conducted to test for the presence of the MON810 and NK603 events in the NK603 x MON810 maize. The presence of the NK603 event was demonstrated and consistent with results using the same restriction enzyme and probe combinations. Similarly, the presence of the MON810 event confirmed the results for the single trait MON810 event. The Southern blot data indicated that gross insert structures and the loci of insertion were retained in the NK603 x MON810 maize. There is therefore no reason to assume any risk from structural modifications to the inserts in the maize. The GMO Panel is of the opinion that the stability of the trait phenotypes also provides evidence that the transgenes are combined as described in the dossier.

2.2.4. Information on the expression of the inserts

The expression levels of CP4 EPSPS and Cry1Ab proteins were measured in grain and forage samples of maize cultivated in field trials at three locations during one season (France in 2000). Cultivated maize lines included NK603 x MON810, NK603 and MON810. In forage, the mean level of the CP4 EPSPS proteins in NK603 x MON810 maize across all sites was 36.3±16.7 μg/g fresh weight as compared to the mean level of 37.2±25.8 μg/g fresh weight from the single trait NK603 maize. In grain samples the levels were 12.7±6.8 μg/g fresh weight and 13.4±4.4 μg/g fresh weight, respectively. The level of CP4 EPSPS was below the limit of detection in MON810.

The mean level of expression of the Cry1Ab protein in forage samples from NK603 x MON810 was 6.06±1.87 μg/g fresh weight compared to a mean level of 6.40±2.62 μg/g fresh weight from the single-trait MON810 transgenic maize. Corresponding amounts in grain samples were 0.73±0.14 and 0.72±0.21 μg/g fresh weight, respectively. The level of Cry1Ab in NK603 was below the limit of detection.

This comparison of the expression levels of CP4 EPSPS and Cry1Ab in grain and forage samples of the same materials (NK603 x MON810, NK603 or MON810) obtained from the different trial sites shows large variations for all three materials and most likely due to natural differences in environmental and agronomic conditions at each site.

The GMO Panel concludes that the level of expression of CP4 EPSPS and Cry1Ab proteins in NK603 x MON810 maize do not raise safety concerns.

2.2.5. Inheritance and stability of inserted DNA

The outcomes of molecular characterisation of the parental lines MON810 and NK603 have been reported in previous opinions (SCP, 1998a; EFSA, 2003a,b) and included an assessment of their genetic stability. Stability was demonstrated as was the segregation of progeny according to Mendelian genetics. Segregation and stability data were consistent with the findings of the Southern blot analyses which demonstrated the stability of the inserted sequences of NK603 and MON810 maize, respectively, and in their progeny. In NK603 x MON810 the two inserts are combined. Southern data show that both events are present and that the structure of each locus remains the same. Furthermore, each of the traits from MON810 and NK603 has been conserved in NK603 x MON810. Thus the GMO Panel is of the opinion that there is no *a priori* reason to expect instability of the transgenes in NK603 x MON810 maize.

2.3. Conclusion

As traditional breeding methods were used in the production of NK603 x MON810 maize, no genetic modification was involved and thus the molecular structures of the DNA inserts are expected to remain unchanged as indicated by the preservation of the phenotypes. Further analysis using Southern blots indicated that insert structures were retained and their genetic stability has been demonstrated in the single events and during the breeding process.

The mean levels of Cry1A(b) and CP4 EPSPS proteins in forage and grain of NK603 x MON810 were not significantly different from the single trait events MON810 and NK603 respectively. There were large variations but similar ranges in the expression of these proteins in NK603 x MON810 and in the individual events NK603 and MON810, respectively. The GMO Panel concludes that these data do not raise safety concerns.

3. Comparative analysis

3.1. Issues raised by the Member States

Questions were raised regarding (1) the compositional analysis of NK603 x MON810 maize and its non genetically modified comparator and (2) the need for a statistical evaluation of data based on at least two growing seasons.

3.2. Evaluation of relevant scientific data

3.2.1. Evaluation of the single events

MON810

In its opinion on MON810 maize under notification C/F/95/12/02, the Scientific Committee on Plants summarized the compositional analysis (SCP, 1998a). Based on the analysis of both forage and kernels of maize MON810 and a non-transgenic control grown during two seasons, this committee concluded that no significant nutritional changes could be detected in maize MON810. The GMO Panel concurs with the opinion of the Scientific Committee on Plants.

NK603

The compositional analysis of maize NK603 was summarized by the GMO Panel in its previous opinion on this single-trait parental line of NK603 x MON810 maize (EFSA, 2003a,b). Compositional data for NK603 maize from two growing seasons revealed a minor, but statistically significant difference for the stearic acid content (C18:0) in kernels compared to non GM maize in one year, but not in the other year. The GMO Panel considered NK603 maize to have the same composition as genetically related non GM maize.

3.2.2. Choice of comparator and production of material for the compositional assessment of NK603 x MON810 maize

NK603 x MON810 maize was compared with a non-transgenic control hybrid (generated from similar, though not identical, inbred parent lines) and with five different non-transgenic commercial maize hybrids. The NK603 x MON810 maize plant itself constituted the F_1 generation and gave material to the forage analysed, whereas self-pollination of the stacked hybrid produced the respective F_2 seed generations, which were the grain material used for the compositional analysis.

Compositional analyses of the NK603 x MON810 maize and its comparator were carried out on forage and grain obtained from three field trials in the European Union, all in France, in year 2000. The GMO Panel considers that these geographical locations are representative for maize growing regions within the EU. These plants were grown under agricultural practices that are typical of maize production within these regions. The herbicide treatments of the maize material used in the compositional comparison depended on its tolerance to glyphosate. Thus, whereas glyphosate was sprayed on glyphosate tolerant NK603 x MON810 maize (and NK603 in field studies in France 2000) to maintain weed control, a set of other herbicides were used to control weeds in the non-glyphosate tolerant comparator and in the conventional maize hybrids.

In this case, where both NK603 and MON810 maize have been assessed in detail by the GMO Panel or are authorised in the EU, the GMO Panel accepts that data for comparative assessment are obtained from one growing season of NK603 x MON810 maize.

3.2.3. Compositional analysis

With regard to composition, grains of NK603 x MON810 maize and its comparator were analyzed for 52 different proximates, nutrients, secondary metabolites and anti-nutrients, including ash, carbohydrates (calculated), acid detergent fibre, neutral detergent fibre, moisture, crude protein, crude total fat, amino acids, fatty acids, vitamin B1, vitamin B2, folic acid, vitamin E, minerals (Ca, Cu, Fe, Mg, Mn, P, K, Na and Zn), phytic acid, trypsin inhibitor, and secondary metabolites (raffinose, inositol, furfural, ferulic acid and p-coumaric acid). Forage was analyzed for 7 components: moisture, ash, crude protein, crude total fat, carbohydrates (calculated), acid detergent fibre, and neutral detergent fibre.

Several statistically significant differences were observed between the level of the studied compounds in NK603 x MON810 maize and its non-transgenic comparator, both when materials harvested at each individual trial site were compared and when combined data were compared. However, differences were usually small and not reproduced at all trial sites, and were generally within the normal variation of conventional maize hybrids. In combined data from all sites on forage, the ash content was higher in NK603 x MON810 than in the control. This difference was only confirmed in one of the three trial sites. In grains, some of the amino acids and fatty acids occurred at higher or lower amounts in NK603 x MON810 than in control material when data from all trial sites were combined, but these altered amounts were not observed at all sites. The only consistent difference in grain was that oleic acid levels were lower in NK603 x MON810 than in the control. When data from all sites were combined, oleic acid

made up 21.6% (20.7-23.3%) of the fatty acids in NK603 x MON810 and 23.3% (21.6-25.4%) in the control. Oleic acid levels in commercial varieties were between 18.1% and 31.9%. In addition to the consistent difference in oleic acid content, differences in amounts of other fatty acids between NK603 x MON810 grain and control grain were sometimes observed but only at some trial sites.

All but one of the significant differences observed for all analysed data were within the 99% tolerance interval (or, in the case where this value was not available, within the range for commercial varieties used in the study) calculated from eleven commercial hybrid reference data of maize grown in the EU, six hybrids from the growing season 1999 and five from the growing season 2000. The statistically significant difference that fell outside the 99% tolerance interval was the phosphorous content in grain. This difference was found at only one of the three field trial sites and was not observed when the entire data was pooled. The difference in phosphorous content of NK603 x MON810 fell just outside the 99% tolerance interval for the commercial varieties in the study, but levels were within the range of historical controls recorded by the applicant and published by Watson in 1987. Field trials in the USA in 2000 and 2001 revealed a similar phosphorous content in NK603 x MON810 grain and its non GM comparators. The evidence does not therefore indicate a cause for concern with regard to the phosphorous content of NK603 x MON810 maize.

As observed differences were small and inconsistent across sites, and fell within the biological variation known for maize, the GMO Panel concludes that the observed differences are minor and not biologically meaningful.

3.2.4. Agronomic traits and GM phenotype

Regarding NK603 x MON810 maize, the GMO Panel does not anticipate interactions (i.e. synergistic or antagonistic) as a result of the genetic modification, which could alter the agronomic characteristics of NK603 x MON810 maize. Furthermore, field trials performed with NK603 x MON810, NK603 and MON810 maize and traditional maize conducted at multiple sites (in Europe and in North America) did not show any phenotypic and agronomic differences except the expected glyphosate-tolerance and insect-resistance. Therefore, the GMO Panel sees no need to ask for additional data on agronomic traits.

3.3. Conclusion

Comparison of the NK603 x MON810 maize with its non-transgenic comparator and various commercial reference hybrids, showed NK603 x MON810 maize to contain the intended transgenic proteins associated with the introduced transgenic traits leading to insect-resistance and glyphosate-tolerance (section 2.2.4). Besides these deliberate changes, this maize showed no relevant alterations in composition, agronomy and phenotype compared with the control line and several conventional maize hybrids. The Panel therefore concludes that the NK603 x MON810 maize is compositionally and phenotypically equivalent to its parental single-trait NK603 and MON810 maize, except for the combination of both traits, and to non-genetically modified maize, except for the introduced traits.

4. Food/feed safety assessment

4.1. Issues raised by the Member States

Questions were raised regarding (1) the limited testing for toxicity and allergenicity of the whole NK603 x MON810 maize, (2) the need for a 90-day sub-chronic toxicity study in rats, (3) the mortality rate of broiler chickens in the feeding study, (4) the possible requirement for testing of

allergenicity in humans, (5) the allergenic potency of the Cry1Ab protein and (6) the need for additional feeding studies with target animals such as ruminants and pigs.

4.2. Evaluation of relevant scientific data

Having considered the information provided in the applications and the Member States comments, the GMO Panel requested from the applicant further information on possible occurrence of residues of glyphosate and its metabolites.

4.2.1. Evaluation of the single events

MON810

Evidence was provided that there is no acute toxicity of the Cry1Ab protein. The results of 90-day sub-chronic rodent studies do not indicate adverse effects from consumption of MON810 maize and therefore confirm that there are no resultant concerns over its safety. For MON810 maize, there are well-performed toxicological studies with the relevant species of animals and a statistically well-designed approach. An allergy risk evaluation of the Cry1Ab was completed, providing indirect evidence for a low probability of allergenicity of the Cry1Ab protein to individuals. The allergenicity of the whole crop does not appear relevant to the GMO Panel since maize is not considered to be a common allergenic food. MON810 maize has been used in nutritional feeding studies with broilers which resulted in no adverse effects. The GMO Panel considers that the nutritional properties of MON810 maize would be no different from those of conventional maize (SCP, 1998a; EFSA, 2004b, c).

NK603

As a result of the genetic modification NK603 maize contains two slightly different CP4 EPSPS proteins expressed from two copies of the *cp4 epsps* (see Section 2.2.2.) gene using different promoters. The proteins differ from each other in one amino acid. Analysis of the impact of this change, besides leading to insensitivity to the herbicide glyphosate (ACNFP, 1994; SCP, 1998a, b: EFSA, 2003a, b), indicated no apparent changes in EPSPS protein structure, activity, toxicity or allergenicity using appropriate bioinformatic approaches, *in vitro* digestion procedures and studies on experimental animals. Furthermore, appropriate animal feeding trials including a 90-day subchronic rodent study indicated that NK603 maize was as safe as its non-GM comparator. Analysis of the grain from field trials in the USA and Europe showed that NK603 maize had the same composition as its non-GM comparator (EFSA, 2003a, b).

4.2.2. Product description and intended use

The GMO Panel assessed the application on NK603 x MON810 maize submitted under Regulation (EC) No 1829/2003 (reference EFSA-GMO-UK-2004-01). This application covers the use of NK603 x MON810 maize as food and feed as for any other maize, i.e. food and feed consisting or derived from the genetically modified maize NK603 x MON810. Maize kernels are used mainly for animal feed and to a smaller scale for direct human consumption i.e. sweet maize kernels. Products from maize kernels such as flour, starch and its by-products gluten, syrups, bran and maize germ oil can be regarded as important base materials for food production.

As the modification in NK603 x MON810 maize is only intended to improve the agronomic performance but not to influence nutritional aspects, production processes and overall use of maize as a crop are not expected to be influenced as a result of the introduction of the GM plants to the market.

4.2.3. Stability during processing

Based on the data of the compositional analysis of the raw agricultural commodities of NK603 x MON810 maize and the non-GM maize comparator, the GMO Panel is of the opinion that there are no reasons to assume that the stability of the processed products derived from NK603 x MON810 maize would be different from the non-GM processed products.

4.2.4. Toxicology

4.2.4.1 Assessment of expressed novel proteins in NK603 x MON810 maize

No new genes in addition to those occurring in the single maize NK603 and MON810 have been introduced in NK603 x MON810 maize.

EPSPS enzymes are natural constituents of maize and other plants, but these plants are sensitive to the action of the herbicide glyphosate. No adverse effects have been linked to the occurrence of these enzymes in maize and other plant foods and feeds. The safety of the CP4 EPSPS and CP4 EPSPS L214P transgenic proteins has previously been assessed for the NK603 maize, on which the GMO Panel previously issued its opinion (EFSA, 2003a, b). In previous evaluations (SCP, 1998a; EFSA, 2004a, b) the safety of the Cry1Ab protein has been shown by testing its *E. coli* equivalent for rapid digestion *in vitro* in simulated gastric fluids and for lack of treatment-related toxicity of the protein in a mouse acute gavage study at dose levels higher that those encountered in human or animal diets.

Given the functional properties of the proteins, the GMO Panel considers that interactions between the expressed proteins are unlikely.

4.2.4.2 Assessment of new constituents other than proteins

As summarized under the section on compositional analysis, no relevant changes have been observed in NK603 x MON810 and therefore no further safety assessment of new constituents in NK603 x MON810 maize is warranted.

4.2.4.3 Toxicological assessment of the whole GM food/feed

Subchronic oral toxicity

NK603 and MON810 maize were tested separately for toxicity as part of the diet for rats in 90-day studies. The results of these 90-day rodent studies do not indicate adverse effects resulting from the consumption of NK603 or MON810 maize.

The GMO Panel accepts that there are valid scientific arguments for the use of data provided for NK603 and MON810 maize for the safety assessment of NK603 x MON810 maize. Given the specific modes of action of the proteins expressed from the inserted *cp4 epsps* and *Cry1Ab* genes, there is no expectation that the CP4 EPSPS and Cry1Ab proteins expressed in these plants would have pleiotropic effects either in isolation or in combination.

Safety of the whole GM food/feed

The genetically modified maize events NK603 and MON810 have previously been found safe for human and animal consumption, and for the environment (EFSA 2003a,b; 2004a; 2005a,b). A molecular characterization undertaken on the NK603 x MON810 maize identified no altered stability of the two events when these were brought together by conventional crossing, and expression analysis of the Cry1Ab and CP4 EPSPS proteins similarly revealed no decisive change in protein expression. Since there are no indications of interactions between the newly

expressed proteins and the composition of NK603 x MON810 maize equivalent to non-GM maize hybrids, the GMO Panel is of the opinion that the NK603 x MON810 maize is as safe as any other conventional maize hybrids. This interpretation is strengthened by a 42-day nutritional feeding study on broiler chicken, which showed NK603 x MON810 maize to be as nutritious as conventional maize. The Panel, therefore, has found no reason to ask for a 90-day toxicology study in rats to confirm the safety of the NK603 x MON810 maize.

4.2.5. Allergenicity

The strategies used when assessing the 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 or not the transformation may have altered the allergenic properties of the modified food. A weight-of-evidence approach is taken by the GMO Panel, taking into account all of the information obtained with various test methods, since no single experimental method yields decisive evidence for allergenicity (EFSA, 2004a; CAC, 2003).

4.2.5.1 Assessment of allergenicity of newly expressed proteins Cry1Ab and CP4 EPSPS

An allergy risk evaluation of CP4 EPSPS and Cry1Ab proteins has been completed using different approaches. From indirect evidence the risk of allergenicity for either protein was determined as being very low. This evidence included the absence of known allergenicity of the source, absence of sequence homology with known allergens and rapid and extensive degradation by pepsin (Metcalfe *et al*., 1996; EFSA, 2004a; CAC, 2003). Previous applications of maize hybrids expressing the CP4 EPSPS or Cry1Ab protein have used the same strategy and have been evaluated by national competent authorities, the EC Scientific Committees and EFSA (SCP, 1998a, b, c; SCP, 2000; SCF, 2002; EFSA, 2003a, b; 2005a, b) and also been approved within the European Community (EC, 1998a,b; EC, 1999; EC, 2004b). The GMO Panel is not aware of any new information on allergenicity that requires a change in this opinion. Also, the GMO Panel is not aware of any new, validated tests that produce additional relevant or accurate information on possible allergenicity of the proteins.

One of the EU Member States mentioned literature on immunogenicity and adjuvanticity of Cry proteins. After intraperitoneal or intragastric administration of Cry1Ac to mice at relatively high dosage, IgG, IgM and mucosal IgA response were induced, but no IgE response was observed (Vazquez-Padron *et al*., 1999a; 2000). This demonstrates that Cry1Ac has no or low allergenic potential. This is also supported by recent bioinformatic studies carried out by the Swedish National Food Administration using a newly developed methodology (Soeria-Atmadja *et al*., 2004; Bjorklund *et al.*, 2005) showing the absence of sequence homology between Cry1Ac and known allergens (unpublished results). On the other hand, Cry1Ab has been shown to act as an adjuvant e.g. it enhances the mucosal and/or the systemic antibody response to a protein which is co-administered with the Cry protein (Vazquez *et al*., 1999b; Moreno-Fierros *et al*., 2003). However the GMO Panel is of the opinion that the adjuvant effect of Cry proteins, observed after high dosage intragastric or intranasal administration will not raise any concerns regarding allergenicity caused by maize consumption or contact. Furthermore, maize is not a common allergenic food, and only a rare cause of occupational allergy may occur.

4.2.5.2 Assessment of allergenicity of the whole GM plant

Risk assessment of the whole GM plant must consider whether allergenicity of the whole crop could be increased as an unintended effect of the random insertion of the transgene in the genome of the host, for example through qualitative or quantitative modification of the pattern of expression of endogenous proteins. Such unintended effects may occur at each genetic modification. However the issue of a possible altered (e.g. increased) allergenicity of the whole GM plant does not appear relevant to the GMO Panel, since maize is not considered a common

allergenic food. Food allergies to maize are of low frequency and mainly occur in populations of specific geographic areas. Rare cases of occupational allergy to corn dust have been reported. There is no reason to expect that the use of GM maize will significantly alter the habit in maize consumption and therefore would not increase the intake and exposure to maize. Therefore a possible overexpression of any endogenous protein, which is not known to be allergenic, would be unlikely to alter the overall allergenicity of the whole plant or the allergy risk for consumers.

4.2.6. Nutritional assessment of GM food/feed

NK603 and MON810 maize have been studied in nutritional feeding studies using rapidly growing day-old broiler chicks, which are widely accepted as a sensitive model to detect nutritional imbalances that might be present in GM maize. Both performance (weight gain, feed consumption) and carcass parameters (weight, weight of carcass parts and compositional analysis of breast and thigh meat) were measured and were similar to that recorded from their near isogenic counterparts. In addition further feeding studies with dairy cows, beef cattle and pigs have been conducted comparing NK603 and MON810 maize to their near isogenic counterparts varieties and commercial hybrids (Erickson *et al*., 2003; Grant *et al*., 2003; Ipharraguerre *et al.*, 2003; Clark and Ipharraguerre, 2001; Donkin *et al.,* 2003; Hyun *et al.*, 2004; Van der Pol *et al.*, 2003). These studies also showed no difference in animal performance, as would be expected from the comparable compositional analyses of these maize varieties. In the view of the GMO Panel, no additional nutritional feeding studies are considered necessary.

A total of 800, Ross-Cobb day-old broiler chicks were used in a 42-day randomised block design study in which there were eight treatment groups. Study diets were formulated to National Research Council (NRC) requirements and contained between 50 and 65% of grain from either NK603 x MON810, a near isogenic counterpart or six commercially available maize hybrids. Animal performance on the diets was evaluated by measuring live weight at day 0 and day 42, total feed intake, feed efficiency and a comprehensive set of carcass measurements. All measurements recorded, including chick mortality (Taylor *et al*., 2003), were similar across diets containing maize grain from NK603 x MON810 maize, the near isogenic counterpart or the six commercial hybrids. The study showed no biologically meaningful differences when comparing the results of the measured parameters for NK603xMON810 maize, the near isogenic counterpart or the six commercial hybrids.

The GMO Panel considers that these data are sufficient to conclude that there is no reason to assume that nutritional properties of maize NK603, MON810 and NK603 x MON810 would be different from those of conventional maize.

4.2.7. Post market monitoring of GM food/feed

NK603 x MON810 maize is, from a nutritional point of view, equivalent to conventional maize. The GMO Panel is of the opinion that a post-market monitoring of the GM food/feed is therefore not regarded necessary.

4.2.8. Residues and metabolites of the herbicide

The issue of a possible occurrence of residues of glyphosate and its metabolite (AMPA) in NK603 x MON810 maize, and the effects of these compounds on animal and human health was raised. The GMO Panel recognizes the importance of the issue and notes that the risk assessment of such compounds is within the scope of Directive 91/414/EEC (EC, 1991).

4.3. Conclusion

Evidence has been provided in previous evaluations that there is no acute toxicity from the CP4 EPSPS and Cry1Ab proteins. The results of 90-day sub-chronic rodent studies in rats with NK603 and MON810, respectively, have been assessed in previous applications by the GMO panel or the Scientific Committee for Plants and do not indicate adverse effects from consumption of NK603 or MON810 maize.

The molecular characterization of NK603 x MON810 revealed no unexpected changes. No interactions between the newly expressed proteins were identified, or any risks from altered allergenicity. In addition, no relevant differences in composition between NK603 x MON810 maize and its appropriate non-transgenic comparators were found. The GMO Panel therefore accepts the applicant's argument that no rodent toxicity study with grains of NK603 x MON810 is required to conclude that NK603 x MON810 maize is as safe for human and animal health as conventional maize.

NK603, MON810, and NK603 x MON810 maize have been studied in separate nutritional feeding studies with broilers and showed no adverse effects. The GMO Panel concludes that the broiler study was also adequate to establish nutritional equivalence and considers that the nutritional properties of maize NK603, MON810 and NK603 x MON810 would be no different from those of conventional maize.

An allergy risk evaluation of the CP4 EPSPS and Cry1Ab proteins was completed, providing indirect evidence for a low probability of allergenicity. A hypothetical altered allergenicity of the whole crop due to the genetic modification does not appear to be relevant to the GMO Panel since maize is not considered a common allergenic food.

5. Environmental risk assessment and monitoring plan

5.1 Issues raised by the Member States

Questions were raised regarding (1) the need to address unintended releases of NK603 x MON810 maize into the environment, (2) the effects of Cry proteins on non-target species and (3) the need for a detailed post market monitoring plan including General Surveillance.

5.2. Evaluation of relevant scientific data

5.2.1 Evaluation of the single events

MON810

The GMO Panel considered the possibility that gene products, particularly Cry proteins might enter the environment either from the intestinal tracts of animals or through horizontal gene transfer to bacteria. Data supplied by the applicant and other literature suggests that most of the Cry1Ab protein would be degraded by enzymatic activity in the intestinal tract so that little Cry toxin would survive to pass out in faeces. There would subsequently be further degradation of proteins in the manure due to microbial processes. Thus amounts of Cry proteins being distributed onto land in manure would be very low minimizing the possibility for exposure of potentially sensitive non-target organisms (*e.g.* soil inhabiting lepidopteran larvae).

There is an issue in that the *cry1Ab* gene in MON810 is synthetic with the aim to produce a changed amino acid sequence in the Cry1Ab protein so as to enhance its toxicity to target insects. The possibility that this synthetic gene could transfer to gut, faecal or soil bacteria such that wild bacteria become transformed to produce this toxin was considered. It is conceivable

that such a gene transfer event would enhance competitiveness or result in ecological impacts in certain environments. Given that marker rescue is established as a possible mechanism for plant to bacterium trans-kingdom DNA transfer, transformation of bacteria already carrying a similar *cry1Ab* toxin gene could be the greatest potential risk. It is well established that DNA is degraded during transit through the gastro-intestinal tract and thus much of the transgenic DNA would be destroyed thereby reducing the possibility for gene exchange with gut, faecal or soil bacteria.

In conclusion, the GMO Panel having considered all the evidence provided was of the opinion that MON810 maize is as safe as conventional maize and therefore the placing on the market of MON810 maize for food, feed, processing and cultivation is unlikely to have an adverse effect on human or animal health or, in that context, on the environment.

NK603

The approved notification C/ES/00/01 for maize NK603 only concerned import and processing. There was therefore no requirement for scientific information on possible environmental effects associated with the cultivation of maize NK603. The GMO Panel agreed with the conclusions of the environmental risk assessment by the applicant that the likelihood of unintended environmental effects due to the adventitious release and spread of NK603 maize will not be different from that of traditionally bred maize. The monitoring plan provided by the applicant was in line with the intended uses for the GMO.

In conclusion, the GMO Panel having considered all the evidence provided was of the opinion that NK603 maize is as safe as conventional maize and therefore the placing on the market of NK603 maize for food, feed and processing is unlikely to have an adverse effect on human or animal health or, in that context, on the environment.

5.3 Environmental risk assessment

5.3.1 Potential unintended effects on plant fitness due to the genetic modification

Both applications cover all uses, excluding cultivation of NK603 x MON810 maize, and thus there is no requirement for scientific information on environmental effects associated with the cultivation. Maize is highly domesticated and not able to survive in the EU environment without cultivation. Maize plants are not winter hardy in many parts of Europe, they have lost their ability to release seeds from the cob and they do not occur outside cultivated land in Europe, despite cultivation for many years. In addition, there are no cross compatible wild relatives in Europe, and gene transfer via pollen is largely restricted to neighbouring crops. Studies in Europe and elsewhere with NK603 and MON810 maize have shown no enhanced weediness or fitness. The environmental risk assessment concludes that the likelihood of unintended environmental effects due to the establishment and spread of NK603 x MON810 maize will not be significantly different from that of NK603 and MON810 and traditionally bred maize. The GMO Panel agrees with this assessment.

5.3.2. Potential for gene transfer

Exposure of microorganisms to transgenic DNA derived from GM maize plants takes place in the environment during natural decay of transgenic plant material. This decay takes place for most GM plant parts in the agricultural areas of the cropped fields, but for pollen this decay may also take place in nearby natural ecosystems. Transgenic DNA is a component of some or most of the food and feed products derived from the GM maize. Therefore microorganisms in the digestive tract of humans and animals feeding on fresh and decaying GM plant material may be exposed to transgenic DNA.

Transgenic pollen is shed and distributed from cultivated GM hybrids or from plants resulting from the adventitious presence of GM kernels in conventionally bred maize seeds. A further but less likely pathway of dispersal of transgenic maize pollen is the flowering of volunteer GM maize plants originating from accidental seed spillage during transport and/or processing. For *Zea mays* any vertical gene transfer is limited to other maize plants as populations of sexually compatible wild relatives of maize are not known in Europe.

(a) Plant to bacteria gene transfer

There is an issue in that the *cry1Ab* gene in MON810 is synthetic with the aim to produce a changed amino acid sequence in the Cry1Ab protein so as to enhance its toxicity to target insects. The possibility that this synthetic gene could transfer to gut, faecal or soil bacteria such that wild bacteria become transformed to produce this toxin was considered. It is conceivable that such a gene transfer event would enhance competitiveness or result in ecological impacts in certain environments. Given that marker rescue is established as a possible mechanism for plant to bacterium trans-kingdom DNA transfer, transformation of bacteria already carrying a similar *cry1Ab* toxin gene could be the greatest potential risk. It is well established that DNA is degraded during transit through the gastro-intestinal tract and thus much of the transgenic DNA would be destroyed thereby reducing the possibility for gene exchange with gut, faecal or soil bacteria. In the very unlikely event of horizontal gene transfer, no principally new trait will be introduced into microbial communities.

(b) Plant to plant gene transfer

Gene transfer can only occur to other maize cultivars and this is considered to be unlikely as NK603 x MON810 maize is not intended for cultivation.

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

The GMO Panel considered the possibility that gene products, particularly Cry proteins might enter the environment either from the intestinal tracts of animals or through horizontal gene transfer to bacteria. Data supplied by the applicant and other literature suggests that most protein would be degraded by enzymatic activity in the intestinal tract so that little Cry toxin would survive to pass out in faeces. There would subsequently be further degradation of proteins in the manure due to microbial processes. Thus amounts of Cry proteins being distributed onto land in manure would be very low minimizing the possibility for exposure of potentially sensitive non-target organisms.

The unintended environmental effects due to adventitious establishment and spread of GM maize will not be different from that of traditionally bred maize. The amounts of Cry toxin being distributed onto land in animal and food waste would be very low, minimizing the possibility for exposure of potentially sensitive non-target organisms (*e.g.* soil inhabiting lepidopteran larvae).

5.3.4. Monitoring

The objectives of a monitoring plan according to Annex VII of Directive 2001/18/EC are to confirm that any assumption regarding the occurrence and impact of potential adverse effects of the GMO, or its use, in the environmental risk assessment are correct and to identify the occurrence of adverse effects of the GMO, or its use, on human health or the environment which were not anticipated in the environmental risk assessment. The scope of the monitoring plan provided by the applicant is in line with the intended uses for the GMO since the environmental risk assessment did not cover cultivation. The GMO Panel advises that appropriate management systems should be in place to restrict seeds of NK603 x MON810 maize entering cultivation, as

the latter requires specific approval under Directive 2001/18/EC or Regulation (EC) No 1829/2003.

5.4. Conclusion

NK603 x MON810 maize is being assessed for import and processing (according to Directive 2001/18/EC) and food/feed uses (according to Regulation (EC) No 1829/2003) only and thus there is no requirement for scientific information on environmental effects associated with cultivation. Maize is highly domesticated and not able to survive in the environment without cultivation. The GMO Panel agrees that unintended environmental effects due to the adventitious establishment and spread of GM maize will be no different to that of traditionally bred maize. The GMO Panel also concludes that the amounts of Cry toxin being distributed onto land in animal and food waste would be very low, minimizing the possibility for exposure of potentially sensitive non-target organisms. Plant to bacteria gene transfer was considered as a negligible risk. The scope of the monitoring plan provided by the applicant is in line with the intended uses for the GMO since the environmental risk assessment did not cover cultivation. The GMO Panel advises that appropriate management systems should be in place to restrict seeds of NK603 x MON810 maize entering cultivation, as the latter requires specific approval under Directive 2001/18/EC or Regulation (EC) No 1829/2003.

CONCLUSIONS AND RECOMMENDATIONS

The GMO Panel assessed the NK603 x MON810 maize which is produced by a cross between inbred lines of maize containing MON810 and NK603 events. The MON810 and NK603 maize events were evaluated previously (SCP, 1998b; EFSA, 2003a,b) and both of them have been authorized for import, processing and food/feed uses (EC, 1998a; EC, 2004b). In assessing the NK603 x MON810 maize, both the single insert lines and the NK603 x MON810 maize were considered. The GMO Panel concluded that it was also acceptable to use data derived from the previous assessments of the single events NK603 and MON810 in support of the safety assessment of the NK603 x MON810 maize.

The GMO Panel, in line with the EFSA Guidance document (EFSA, 2004a), has assessed the molecular characterisation of the combined traits in NK603 x MON810 together with data on the levels of Cry1Ab and CP4 EPSPS protein expression and compositional analysis. Comparisons on expression were made using the single events MON810 and NK603.

The GMO Panel has reached the opinion that NK603 x MON810 maize is unlikely to have adverse effects on human and animal health. NK603 x MON810 maize is being assessed for import for food and feed uses only and thus there is no requirement for scientific information on environmental effects associated with cultivation. However, The GMO Panel agrees that in the case of any adventitious establishment, any unintended environmental effects due to spread of NK603 x MON810 maize will not be significantly different to that of traditionally bred maize.

In conclusion, the GMO Panel considers that information available for NK603 x MON810 maize addresses the outstanding questions raised by the Member States and considers it unlikely that NK603 x MON810 maize will have any adverse effect on human and animal health or the environment in the context of its proposed uses.

DOCUMENTATION PROVIDED TO EFSA

1. Letter from the UK Competent Authority (Food Standards Agency), dated 8 June 2004 concerning the submission to EFSA of application for authorization of NK603 x MON810 in accordance with Regulation (EC) 1829/2003.

2. Letter from EFSA to applicant with request for clarification/additional information (ref. SR/MR/sp (2004) 538, 28 July 2004).
3. Letter from the applicant, dated 8 February 2005, providing a revised application to EFSA.
4. Letter from EFSA to applicant with request for clarification/additional information (ref. SR/AC/jq (2005) 427, 13 April 2005).
5. Letter from the applicant, dated 9 June 2005, providing EFSA with a second revision of the application.
6. Letter from EFSA to applicant, dated 17 June 2005, concerning the 'Statement of Validity' for application EFSA/GMO/UK/2004/01, NK603 x MON810 maize submitted under Regulation (EC) 1829/2003 (ref. SR/SM/jq (2005) 750).
7. Submission of the application EFSA/GMO/UK/2004/01 by Monsanto to EFSA, containing:

   Part I – technical dossier
   Part II – summary
   Part III – Carthagena Protocol
   Part IV – labelling proposal
   Part V – samples and detection method
   Part VI – additional information for GMOs

8. Notification C/GB/02/M3/3 from Monsanto on the hybrid maize NK603 x MON810, including the technical dossier and annexes (e.g. summary, assessment report carried out by the lead competent authority, additional data) as well as the respective Member States comments/objections.

REFERENCES

ACNFP, 1994. Advisory Committee on Novel Foods and Processes, UK. (line 408, page 11).

ACNFP, 1996. Advisory Committee on Novel Foods and Processes, UK.

Björklund, A.K., Soeria-Atmadja, D., Zorzet, A., Hammerling, U. & Gustafsson, M.G., 2005. Supervised identification of allergen-representative peptides for in silico detection of potentially allergenic proteins. Bioinformatics, 21, 39-50.

CAC, 2003. Codex principles and guidelines on foods derived from biotechnology. Joint FAO/WHO Food Standards Programme, Food and Agriculture Organisation, Rome. ftp://ftp.fao.org/codex/standard/en/CodexTextsBiotechFoods.pdf

Clark, J.H. and Ipharraguerre, I.R., 2001. Livestock performance: Feeding biotech crops. J. Diary Sci., 84 (E Supp.), E9-E-18.

Donkin, S.S., Velez, J.C., Totten, A.K., Stanisiewski, E.P. and Hartnell, G.F., 2003. Effects of feeding silage and grain from glyphosate-tolerant or insect-protected corn hybrids on feed intake, ruminal digestion, and milk production in dairy cattle. J. Dairy Sci., 86, 1780-1788.

EC, 1990. Council Directive 90/220/EC of 23 April 1990 on the deliberate release into the environment of genetically modified organisms. OJ L 117, 15–27. http://europa.eu.int/smartapi/cgi/sga_doc?smartapi!celexapi!prod!CELEXnumdoc&lg=EN&numdoc=31990L0220&model=guichett

EC, 1991. Council Directive 91/414/EEC of 15 July 1991 concerning the placing of plant protection products on the market. OJ L230, 1-32. http://europa.eu.int/smartapi/cgi/sga_doc?smartapi!celexapi!prod!CELEXnumdoc&lg=EN&numdoc=31991L0414&model=guichett

EC, 1997. Regulation (EC) No 258/97 of the European Parliament and of the Council of 27 January 1997 concerning novel foods and novel food ingredients. OJ L43, 1-6. http://europa.eu.int/smartapi/cgi/sga_doc?smartapi!celexapi!prod!CELEXnumdoc&lg=EN&numdoc=31997R0258&model=guichett

EC, 1998a. Commission Decision 98/294/EC of 22 April 1998 concerning the placing on the market of genetically modified maize (*Zea mays* L. line MON810), pursuant to Council Directive 90/220/EEC. OJ L131, 32-33. http://europa.eu.int/eur-lex/pri/en/oj/dat/1998/l_131/l_13119980505en00320033.pdf

EC, 1998b. Commission Decision 98/292/EC of 22 April 1998 concerning the placing on the market of genetically modified maize (*Zea mays* L. line Bt11), pursuant to Council Directive 90/220/EEC. OJ L131, 28-29. http://europa.eu.int/eur-lex/pri/en/oj/dat/1998/l_131/l_13119980505en00280029.pdf

EC, 1999. Summary of notification received in 1998 by the Commission pursuant to Article 5 of Regulation (EC) 258/97 of the European Parliament and of the Council. http://europa.eu.int/eur-lex/pri/en/oj/dat/1999/c_181/c_18119990626en00220022.pdf

EC, 2001. Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC. OJ L106, 1-39. http://europa.eu.int/eur-lex/pri/en/oj/dat/2001/l_106/l_10620010417en00010038.pdf

EC, 2002. Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L31, 1-24. http://europa.eu.int/eur-lex/pri/en/oj/dat/2002/l_031/l_03120020201en00010024.pdf

EC, 2003. Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed. OJ L268, 1-23. http://europa.eu.int/eur-lex/pri/en/oj/dat/2003/l_268/l_26820031018en00010023.pdf

EC, 2004a. Notification pursuant to Article 5 of Regulation (EC) N° 258/97 of the European Parliament and the Council. http://europa.eu.int/comm/food/food/biotechnology/novelfood/notif_list_en.pdf

EC, 2004b. Commission Decision 2004/643/EC 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 (notified under document number C(2004) 2761). OJ L295, 35. http://europa.eu.int/eur-lex/pri/en/oj/dat/2004/l_295/l_29520040918en00350037.pdf

EC, 2005. Commission Decision 2005/448/EC of 3 March 2005 authorising the placing on the market of foods and food ingredients derived from genetically modified maize line NK603 as novel foods or novel food ingredients under Regulation (EC) No 258/97 of the European Parliament and of the Council. OJ L158, 20. http://europa.eu.int/eur-lex/lex/LexUriServ/site/en/oj/2005/l_158/l_15820050621en00200022.pdf

EFSA, 2003a. Opinion of the Scientific Panel on Genetically Modified Organisms on a request from the Commission related to the safety of foods and food ingredients derived from herbicide-tolerant genetically modified maize NK603, for which a request for placing on the market was submitted under Article 4 of the Novel Food Regulation (EC) No 258/97 by Monsanto (Question No EFSA-Q-2003-002). EFSA Journal 9, 1-14. http://www.efsa.eu.int/science/gmo/gmo_opinions/177/opinion_gmo_02_final_en1.pdf

EFSA, 2003b. Opinion of the Scientific Panel on Genetically Modified Organisms on a request from the Commission related to the Notification (Reference CE/ES/00/01) for the placing on the market of herbicide-tolerant genetically modified maize NK603, for import and processing, under Part C of Directive 2001/18/EC from Monsanto. EFSA Journal 10, 1-13. http://www.efsa.eu.int/science/gmo/gmo_opinions/176_en.html

EFSA, 2004a. Guidance document of the Scientific Panel on Genetically Modified Organisms for the Risk Assessment of Genetically Modified Plants and Derived Food and Feed. EFSA Journal 99, 1-94. http://www.efsa.eu.int/science/gmo/gmo_guidance/660_en.html

EFSA, 2004b. Opinion of the Scientific Panel on Genetically Modified Organisms on a request from the Commission related to the Notification (Reference C/DE/02/9) for the placing on the market of insect protected genetically modified maize MON 863 and MON 863 x MON810, for import and processing, under Part C of Directive 2001/18/EC from Monsanto. EFSA Journal 49, 1-25. http://www.efsa.eu.int/science/gmo/gmo_opinions/381/opinion_gmo_06_en1.pdf

EFSA, 2004c. Opinion of the Scientific Panel on Genetically Modified Organisms on a request from the Commission related to the safety of foods and food ingredients derived from insect-protected genetically modified maize MON 863 and MON 863 x MON810, for which a request for placing on the market was submitted under Article 4 of the Novel Food Regulation (EC) No 258/97 by Monsanto. EFSA Journal 50, 1-25. http://www.efsa.eu.int/science/gmo/gmo_opinions/383/opinion_gmo_07_en1.pdf

EFSA, 2005a. Opinion of the Scientific Panel on Genetically Modified Organisms on a request from the Commission related to the notification (Reference C/ES/01/01) for the placing on the market of insect-tolerant genetically modified maize 1507, for import, feed and industrial processing and cultivation, under Part C of Directive 2001/18/EC from Pioneer Hi-Bred International/Mycogen Seeds. EFSA Journal 181, 1-33. http://www.efsa.eu.int/science/gmo/gmo_opinions/catindex_en.html

EFSA, 2005b. Opinion of the Scientific Panel on Genetically Modified Organisms on an application (Reference EFSA-GMO-BE-2004-07) for the placing on the market of insect-protected glyphosate-tolerant genetically modified maize MON863 x MON810 x NK603, for food and feed uses, and import and processing under Regulation (EC) No 1829/2003 from Monsanto. EFSA Journal 256, 1-25.
http://www.efsa.eu.int/science/gmo/gmo_opinions/1033_en.html

EFSA, 2005c. Opinion of the Scientific Panel on Genetically Modified Organisms on a request from the Commission related to the notification (Reference C/GB/02/M3/3) for the placing on the market of glyphosate-tolerant and insect-resistant genetically modified maize NK603 x MON810, for import and processing, under Part C of Directive 2001/18/EC from Monsanto. EFSA Journal 308, 1-22.
http://www.efsa.eu.int/science/gmo/gmo_opinions/catindex_en.html

Erickson, G.E., Robins, N.D., Simon, J.J., Berger, L.L., Klopfenstein, T.J., Stanisiewski, E.P. and Hartnell, G.F., 2003. Effect of feeding glyphosate-tolerant (Roundup –Ready events GA21 or NK603) corn compared with reference hybrids on feedlot steer performance and carcass characteristics. J. Anim. Sci., 81, 2600-2608.

Grant, R.J., Fanning, K.C., Kleinschmit, D., Stanisiewski, E.P. and Hartnell, G.F., 2003. Influence of glyphosate-tolerant (event nk603) and corn rootworm protected (event MON863) corn silage and grain on feed consumption and milk production in Holstein cattle. J. Diary Sci., 86, 1707-1715.

Hyun, Y., Bressner, G.E., Ellis M., Lewis A.J., Fisher R., Stanisiewski E.P., Hartnell G.F. (2004). Performance of growing-finishing pigs fed diets containing Roundup Ready corn (event NK603), a nontransgenic genetically similar corn, or conventional corn lines. J. Animal Sci., 82, 571-580.

Ipharraguerre, I.R., Younker, R.S:, Clark, J.H., Stanisiewski, E.P. and Hartnell, G.F., 2003. Performance of lactating dairy cows fed corn as whole plant silage and grain produced from a glyphosate-tolerant hybrid (event NK603). J. Dairy Sci., 86, 1734-1741.

Metcalfe, D.D., Astwood, J.D., Townsend, R., Sampson, H.A., Taylor, S.L. and Fuchs, R.L., 1996. Assessment of the allergenic potential of foods derived from genetically engineered crop plants. Crit. Rev. Food. Sci. Nutr., 36(S), 165-186.

Moreno-Fierros, L., Ruiz-Medina, E.J., Esquivel, R., López-Revilla, R. & Piña-Cruz, S., 2003. Intranasal Cry1Ac protoxin is an effective mucosal and systemic carrier and adjuvant of Streptococcus pneumoniae polysaccharides in mice. Scand. J. Immunol., 57, 45-55.

SCF, 2002. Opinion on a request to place genetically modified sweet maize line Bt11 on the market (expressed on 17 April 2002).
http://europa.eu.int/comm/food/fs/sc/scf/out129_en.pdf

SCP, 1998a. Opinion of the Scientific Committee on Plants regarding the genetically modified, insect resistant maize lines notified by the Monsanto Company.
http://europa.eu.int/comm/food/fs/sc/scp/out02_en.html

SCP, 1998b. Opinion of the Scientific Committee on Plants regarding submission for placing on the market of genetically modified, insect-resistant maize lines notified by the Pioneer Genetique S.A.R.L. Company (notification No C/F/95/12-01/B).
http://europa.eu.int/comm/food/fs/sc/scp/out10_en.html

SCP, 1998c. Opinion of the Scientific Committee on Plants on the Genetically Modified Maize Lines Notified by the Novartis Company (Notification C/GB/96/M4/1). http://europe.eu.int/comm/food/fs/sc/scp/out05_en.html

SCP, 2000. Opinion of the Scientific Committee on Plants on the submission for placing on the market of genetically modified insect resistant and glufosinate ammonium tolerant (Bt-11) maize for cultivation. Notified by Novartis Seeds SA Company (notification C/F/96/05-10). http://europa.eu.int/comm/food/fs/sc/scp/out86_gmo_en.html

Soeria-Atmadja, D., Zorzet, A., Gustafsson, M &Hammerling, U., 2004. Statistical evaluation of local alignment features for prediction of allergenicity using supervised classification algorithms. International Archives of Allergy and Immunology, 133, 101-112.

Van der Pol, K., Simon, J., Erickson, G., Klopfenstein, T., Stanisiewski, E., and Hartnell, G.F., 2003. Feeding transgenic (Bt corn rootworm protected and Roundup Ready®) corn to feedlot cattle. Neb. Beef Rep. MP, 80-A, 30-32.

Vazquez-Padron, R.I., Moreno-Fierros, L., Neri-Bazan, L., De la Riva, G.A. & Lopez-Revilla, R., 1999a. Intragastric and intraperitoneal administration of Cry1Ac protoxin from Bacillus thuringiensis induce systemic and mucosal immune response in mice. Life Sciences, 64, 1897-1912.

Vazquez-Padron, R.I., Moreno-Fierros, L., Neri-Bazan, L., de la Riva, G.A. & Lopez-Revilla, R., 1999b. Bacillus thuringiensis Cry1Ac protoxin is a potent systemic and mucosal adjuvant. Scand. J. Immunol., 49, 578-584.

Vazquez-Padron, R.I., Moreno-Fierros, L., Neri-Bazan, L., Martinez-Gil, A.F., De la Riva, G.A. & Lopez-Revilla, R., 2000. Characterization of the mucosal and systemic immune response induced by Cry1Ac protein from Bacillus thuringiensis HD 73 in mice. Braz. J. Med. Biol. Res., 33, 147-55.

Watson, S.A. (1987). Corn marketing, processing and utilisation. In: Sprague, G.F. and Dudley, J.W. (eds.) Corn and Corn Improvement. American Society of Agronomy, Madison, Wisconsin, 885–940.

Taylor, M. L., Hartnell, G. F., Riordan, S. G., Nemeth, M. A., Karunanandaa, K., George, B. and Astwood, J. D., 2003. Comparison of Broiler Performance When Fed Diets Containing Grain from Roundup Ready (NK603), YieldGard × Roundup Ready (MON810 × NK603), Non-transgenic Control, or Commercial Corn1. Poultry Sci., 82, 443-453.

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