

**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<sup>1</sup>**

**(Question No EFSA-Q-2004-159)**

**Opinion adopted on 6 July 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 MON 863 x MON 810 x NK603 (Unique Identifier MON-00863-5 x MON-00810-6 x MON-00603-6) to provide protection against specific lepidopteran and coleopteran pests and tolerance to the herbicide glyphosate.

In delivering its opinion the Panel considered the application, additional information provided by the applicant and comments submitted by the Member States. Further information from applications for placing the single insert lines on the market under EU regulatory procedures was taken into account where appropriate.

MON 863 x MON 810 x NK603 maize was assessed with reference to the intended uses and the appropriate principles described in the guidance document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants and derived food and feed. The scientific assessment included molecular characterisation of the inserted DNA and expression of target proteins. A comparative analysis of agronomic traits and composition was undertaken and the safety of the new proteins and the whole food/feed was evaluated with respect to toxicity and allergenicity. Both a nutritional and an environmental assessment, including monitoring plans, were undertaken.

MON 863 maize was developed to provide protection against certain coleopteran pests, principally corn rootworm (*Diabrotica* spp.) by the introduction of a variant *Bacillus thuringiensis cry3Bb1* gene expressing an insecticidal protein. MON 863 has received an EFSA opinion in favour of its authorisation. MON 810 maize produces the protein Cry1Ab, which confers protection against certain lepidopteran insect pests (*Ostrinia nubilalis* and *Sesamia* spp.). MON 810 was approved under Directive 90/220/EEC by Commission Decision 98/294/EC. The use of food ingredients from MON 810 maize was notified in 1997 under Regulation (EC) No 258/97. NK603 was developed to be tolerant to the herbicide glyphosate by the introduction of a gene coding for 5-enolpyruvylshikimate-3-phosphate synthase from *Agrobacterium* sp. strain CP4 (CP4 EPSPS). NK603 has received an opinion in favour of its authorisation and was authorised under Directive 2001/18/EC by Commission Decision 2004/643/EC. The use of food

---

<sup>1</sup> For citation purposes: 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 MON 863 x MON 810 x NK603, for food and feed use, import and processing under Regulation (EC) No 1829/2003 from Monsanto, The EFSA Journal (2005) 256, 1-25.

and food ingredients from NK603 maize were authorised under the Regulation (EC) No 258/97 by Commission Decision 2005/448/EC.

MON 863 x MON 810 x NK603 maize was produced by crosses between maize inbred lines containing MON 863, MON 810 and NK603 events to combine the rootworm resistance trait in MON 863 with the trait present in MON 810 protecting against lepidopteran pests and the tolerance to the herbicide glyphosate in NK603.

Molecular analysis of the DNA inserts present in the MON 863 x MON 810 x NK603 maize confirmed that the insert structures of the single events were retained.

Cry3Bb1, Cry1Ab and CP4 EPSPS protein levels in kernels of the MON 863 x MON 810 x NK603 maize were comparable to the individual MON 863, MON 810 and NK603 lines.

The safety of the Cry3Bb1, Cry1Ab, CP4 EPSPS and NptII proteins has previously been assessed in the single events for which positive opinions were issued.

MON 863 x MON 810 x NK603 maize was found to contain transgenic proteins associated with the introduced transgenic traits of insect- and herbicide-resistance. Besides these deliberate changes, this maize showed no marked alterations in composition, agronomy and phenotype compared with the control lines and reference lines. The Panel therefore concludes that MON 863 x MON 810 x NK603 maize is compositionally and phenotypically equivalent to its parental single-trait GM lines and non-genetically modified maize, except for the introduced traits.

A 90-day sub-chronic rodent study with MON 863 x MON 810 x NK603 maize indicated that there are no adverse effects from its consumption.

Feeding studies conducted on broilers with MON 863 x MON 810 x NK603 maize showed no adverse effects. The Panel considers that the nutritional properties of this maize would be no different from those of conventional maize.

The application EFSA-GMO-BE-2004-07 concerns food and feed uses and import and processing. There is therefore no requirement for scientific information on possible environmental effects associated with the cultivation of the maize lines. The GMO Panel agrees that unintended environmental effects due to the establishment and spread of GM maize will not be different from that of traditionally bred maize. The monitoring plan provided by the applicant is in line with the intended uses for the GMO.

In conclusion, the Panel considers that the information available for MON 863 x MON 810 x NK603 maize addresses the outstanding questions raised by the Member States and considers that it will not have adverse effects on human and animal health or the environment in the context of its proposed use.

**Key words:** GMOs, maize, MON 810, MON 863, NK603, MON 863 x MON 810 x NK603, MON-00863-5 x MON-00810-6 x MON-00603-6, insect protection, glyphosate resistance, Cry3Bb1, Cry1Ab, NptII, CP4 EPSPS, food safety, feed safety, human health, environment, import, Directive 2001/18/EC, Regulation (EC) No 1829/2003.

## TABLE OF CONTENTS

SUMMARY.....	1
BACKGROUND.....	3
TERMS OF REFERENCE.....	4
ASSESSMENT.....	5
CONCLUSIONS AND RECOMMENDATIONS.....	20
DOCUMENTATION PROVIDED TO EFSA.....	20
REFERENCES.....	21
SCIENTIFIC PANEL MEMBERS.....	25
ACKNOWLEDGEMENT.....	25

## BACKGROUND

On 23 November 2004 EFSA received from the Belgian Competent Authority an application (Reference EFSA-GMO-BE-2004-07), for authorisation of MON 863 x MON 810 x NK603 maize (Unique Identifier MON-00863-5 x MON-00810-6 x MON-00603-6), 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-BE-2004-07 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<sup>2</sup>. 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 14 January 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 14 April 2005) within which to make their opinion known.

On 27 April 2005 the GMO Panel asked for data on the existing 90-days rat study with MON 863 x NK603, which it considered appropriate to evaluate. The applicant provided the study on 19 May 2005. After receipt of the full data package, the GMO Panel finalised its risk assessment of MON 863 x MON 810 x NK603 maize.

The Scientific Panel on Genetically Modified Organisms carried out a scientific assessment of the genetically modified maize MON 863 x MON 810 x NK603 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 and the additional information provided. Further information from applications for placing the single insert lines on the market under EU regulatory procedures was taken into account where appropriate.

---

<sup>2</sup> [http://www.efsa.eu.int/science/gmo/gm\\_ff\\_applications/catindex\\_en.html](http://www.efsa.eu.int/science/gmo/gm_ff_applications/catindex_en.html)

The single events MON 863, MON 810 and NK603 have been the subjects of earlier assessments. MON 863 and NK603 have received an EFSA opinion in favour of their authorisation (EFSA, 2004a,b; 2003a,b). MON 810 was approved under Directive 90/220/EEC by Commission Decision 98/294/EC (EC, 1998a). The use of food ingredients from MON 810 maize was notified in 1997 under Regulation (EC) No 258/97<sup>3</sup> (EC, 1998b). NK603 was authorised under Directive 2001/18/EC by Commission Decision 2004/643/EC (EC, 2004a). The use of food and food ingredients from NK603 maize was authorised under the 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. As additional information was requested by EFSA GMO Panel, the time-limit of 6 months was extended accordingly in line with Articles 6(1), 6(2) and Articles 18(1), 18(2) of Regulation (EC) No 1829/2003. Currently, the JRC-CRL is still awaiting additional information from the applicant to comply with Articles 6(5)(f) and 18(5)(f) of that Regulation.

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.

## 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 MON 863 x MON 810 x NK603 for food and feed uses, import and processing.

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 Panel was not requested to give an opinion on information required under Annex II to the Cartagena Protocol. The 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.

---

<sup>3</sup> 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 MON 810' was considered by the UK Advisory Committee on Novel Foods and Processes (ACNFP, 1996).

## ASSESSMENT

### 1. Introduction

The GM maize MON 863 x MON 810 x NK603 is assessed with reference to its intended use and the appropriate principles described in the guidance document of the GMO Panel for the risk assessment of genetically modified plants and derived food and feed (EFSA, 2004c). MON 863 x MON 810 x NK603 maize might be regarded as a separate GM plant construct or an example of extended use of the component single insert lines MON 863, MON 810 and NK603. This distinction has no bearing on the scientific assessment that was undertaken by the Panel and the conclusions are relevant in either case. Throughout the document the GM hybrids are referred to as MON 863 x MON 810 x NK603 maize. The combination of separate inserts as a result of a cross between GM plants raises questions about the extent to which data on the individual GM plant lines can be extrapolated to assess MON 863 x MON 810 x NK603 maize. The Panel regards this as a case-by-case issue in which the detail of the individual inserts is of particular relevance.

### 2. Molecular characterisation

#### 2.1. Issues raised by Member States

(1) Comments were made regarding the structure and stability of the insert in MON 863 x MON 810 x NK603 maize; (2) comments were made regarding the differences in expression of the Cry proteins in MON 863 x MON 810 x NK603 maize and parental transgenic lines and the variability between different locations; (3) the presence of the *nptII* antibiotic resistance gene was raised.

#### 2.2. Evaluation of relevant scientific data

The EFSA GMO Panel guidance document (EFSA, 2004c) 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 lines 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 MON 863 x MON 810 x NK603 maize and no new genetic manipulation was involved. The three transgenic inserts that are present in MON 863 x MON 810 x NK603 maize were derived from three independent single trait GM maize lines: MON 863, MON 810 and NK603. Each of these GM maize lines was the subject of an earlier safety evaluation and separate Opinions for each of them have been published (EFSA, 2004a,b; SCP 1998b; EFSA, 2003a,b). The maize MON 863 x MON 810 x NK603 combines the insect protection traits from MON 863 and MON 810 with the herbicide 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.

The use of GM maize in agriculture involves analogous traditional breeding methods for the production of hybrid seed and this is the case both for GM maize with a single transgenic insert and for multiple trait hybrids, including MON 863 × MON 810 × NK603.

Where two GM traits are combined in a hybrid the individual inbred GM lines can be crossed as a final stage in the production of seed for agriculture. The breeding process is necessarily more complex in the case of MON 863 × MON 810 × NK603 maize where three independent transgenic traits are combined. The applicant uses three related breeding strategies to produce MON 863 × MON 810 × NK603 seed. Inbred lines are produced for each of the three single trait GM maize lines: MON 863, MON 810 and NK603. Inbred lines are produced for the three possible paired combinations of the independent transgenic inserts: MON 863 × MON 810, MON 810 × NK603 and MON 863 × NK603. The final stage in MON 863 × MON 810 × NK603 seed production involves a cross between appropriate inbred lines with a single and a double transgenic insert. The resulting seed is used in agriculture.

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

#### MON863

Genetically modified maize MON 863 was developed to produce an insecticidal activity against corn rootworm by the introduction of a *Bacillus thuringiensis* gene encoding an insecticidal variant Cry3Bb1 protein. The variant Cry3Bb1 protein expressed in MON 863 maize has seven amino acid differences from wild type Cry3Bb1 and was designed to enhance its expression in plants and its insecticidal activity against corn rootworm. Particle acceleration was used to introduce a *MluI* restriction fragment isolated from the bacterial plasmid vector PV-ZMIR13. This fragment contained a selectable marker gene *nptII* encoding neomycin phosphotransferase II and the trait gene encoding a variant *Bacillus thuringiensis* Cry3Bb1 insecticidal protein (Crickmore et al., 1998).

For MON 863 maize, detailed molecular analysis demonstrated that only the two expected full length proteins, Cry3Bb1 and NptII, would be encoded by the insert. The GMO Panel recently concluded that the use of the *nptII* gene as a selectable marker did not pose a risk to the environment or to human and animal health (see section 5.2.2.2 of this opinion; EFSA, 2004d and references therein). Nucleotide sequences at the junctions between the insert and parental DNA were determined and bioinformatic analysis revealed the presence of mitochondrial DNA at both the 5' and 3' flanks. The integration of organellar DNA within the nuclear plant genome – being already present or acquired during the transformation- is established as a normal phenomenon in plant biology and the Panel considered that this would not significantly impact on the present safety assessment. A bioinformatic analysis of DNA sequences spanning the 5' and 3' junctions of the insert was undertaken. Identified open reading frames were analyzed to test for the creation of a potential peptide with homology to known allergens, toxins or proteins that display adverse health effects and these were not found. The genetic stability of the inserted DNA in event MON 863 was demonstrated by Southern blot analysis of genomic DNA from nine plant generations and segregation data for the Cry3Bb1 trait was studied using Chi square analysis of Mendelian inheritance data over five generations (EFSA, 2004a,b and references therein).

#### MON810

GM maize MON 810 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 and subsequent molecular characterization demonstrated that the sequences actually inserted included sufficient of the *cry1Ab* coding region to encode an insecticidal Cry1Ab protein (SCP, 1998b).

The maize line MON 810 was the subject of an earlier safety assessment (Notification C/F/95/12-02; SCP, 1998b) 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 MON 810 was determined and this confirmed its predicted structure. This consists of the enhanced CaMV 35S promoter, the maize HSP70 intron and that 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 MON 810 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 MON 810 was resolved (EFSA, 2004a,b).

### NK603

The maize line NK603 was the subject of an earlier safety assessment (EFSA, 2003a,b). In the NK603 event glyphosate tolerance was achieved by the introduction of a gene encoding glyphosate tolerant 5-enolpyruvylshikimate-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 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. Moreover, the toxicological assessment does not indicate adverse effects from consumption of maize NK603 (see section 4.2.1).

### 2.2.3. Transgenic constructs in the hybrid

Crosses were used to construct the maize MON 863 × MON 810 × NK603. The molecular structures of the DNA inserts present in this maize were investigated using Southern analyses. The presence of the MON863 insert was confirmed by *EcoRV* digestion and hybridisation with a *cry3Bb1* probe. The expected 10kb restriction fragment was detected. This represents part of the insert DNA and one of the flanks that are characteristic of event MON863. The presence of the MON810 insert was confirmed by digestion with *EcoRI* and *NcoI* and hybridisation with a *cry1Ab* probe. The expected 2.8kb restriction fragment was detected. A fragment size inconsistency in the dossier was resolved by reference to the insert DNA sequence for event NK603. This represents part of the insert DNA and one of the flanks that are characteristic of in event MON810. The presence of the NK603 insert was confirmed by *EcoRV* digestion and hybridisation with a *ctp2-cp4-epsps* probe. The expected 2.8kb and 3.8kb restriction fragments were detected. This represents the majority of the insert DNA that is present in event NK603. This additional analysis confirmed that the insert structures were retained in this maize. The continued presence of the trait phenotypes also provides evidence for the stability of the transgenes in the combined maize as described in the dossier.

### 2.2.4. Information on the expression of the insert

The expression levels of the Cry1Ab, Cry3Bb1, CP4 EPSPS, and NptII proteins were measured by an immunochemical assay. Cry1Ab, Cry3Bb1 and CP4 EPSPS, but not NptII, occurred at

detectable levels in kernels. Cry1Ab levels (average plus minus standard deviation) were  $0.65 \pm 0.08$   $\mu\text{g/g}$  dw (dry weight) in MON 863 x MON 810 x NK603 and  $0.77 \pm 0.09$   $\mu\text{g/g}$  dw in MON 810. The levels of Cry3Bb1 in kernels were  $37 \pm 11$   $\mu\text{g/g}$  dw in MON 863 x MON 810 x NK603 and  $29 \pm 3.9$   $\mu\text{g/g}$  DW in MON 863. Those of CP4 EPSPS were  $11 \pm 2.8$   $\mu\text{g/g}$  dw in MON 863 x MON 810 x NK603 and  $12 \pm 1.8$   $\mu\text{g/g}$  dw in NK603. Taking into account the range of values obtained for each of the event under field conditions, the expression levels of these proteins in MON 863 x MON 810 x NK603 are comparable with those in MON 863, MON 810 and NK603.

### 2.2.5. Inheritance and stability of inserted DNA

The genetic stability of the inserted DNA in events MON 863, MON810 and NK603 was demonstrated in earlier safety evaluations. In MON 863 x MON 810 x NK603 the three inserts are combined. The Southern data presented show that all three events are present and the structure of each insert is retained. Furthermore, each of the traits has been conserved in the combined maize line. The Panel agreed that there is no *a priori* reason to expect instability of stacked transgenes.

The assessment of stability with respect to individual transgenic inserts has been undertaken already and to assess stability of the stacked events is irrelevant where the stack is the final cross in the development of hybrid seed. Effectively the F1 hybrid is sown and the F2 generation of seed is used as food or feed. A strategy for hybrid maize production might involve the development of an inbred line carrying stacked transgenes in which case this would be crossed with a non-GM inbred line to generate hybrid maize seed. In such a case the stacked transgenes are maintained through several generations making an assessment of stability feasible. Where three transgenic events are stacked the use of an inbred line carrying two transgenes might be crossed with another inbred GM line carrying the third transgene to produce hybrid seed. The Panel is agreed that there is no *a priori* reason to expect instability of stacked transgenes.

### 2.3. Conclusion

As traditional breeding methods were used in the production of the maize MON 863 x MON 810 x NK603, 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 levels of expressed transgenic proteins in MON 863 x MON 810 x NK603 maize and the parental GM maize, *i.e.* Cry3Bb1 in MON 863 x MON 810 x NK603 and MON 863, Cry1Ab in MON 863 x MON 810 x NK603 and MON 810, as well as CP4 EPSPS in MON 863 x MON 810 x NK603 and NK603, were measured. Taking into account the range of values obtained for each of the event under field conditions, the expression levels of these proteins in MON 863 x MON 810 x NK603 are comparable with those in MON 863, MON 810 and NK603. In most samples, the NptII transgenic protein was undetectable in kernels of maize MON 863 x MON 810 x NK603 and MON 863.

The Panel concludes that these data do not raise safety concerns.

### **3. Comparative analysis**

#### **3.1. Issues raised by Member States**

The analysis of compositional and agronomic/phenotypic data from additional geographical regions and growing seasons was suggested.

#### **3.2. Evaluation of relevant scientific data**

##### **3.2.1. Evaluation of the single events**

In its previous opinion on maize MON863, the Panel summarized the comparative compositional analyses of MON 863 grown during two seasons (EFSA, 2004a,b). Macro-nutrients, micro-nutrients, and anti-nutrients, as well as secondary metabolites were measured. Some statistically significant but small differences were observed for palmitic acid (C16:0) between MON 863 and its control. These differences were within the historical background range. The Panel thus concluded that these differences were of no biological significance.

The compositional analysis of maize NK603 was also summarized by the Panel in its previous opinion on this single-trait parental line of MON 863 x MON810 x NK603 maize (EFSA, 2003a,b). Compositional data for maize NK603 from two growing seasons revealed a minor, but statistically significant difference for the stearic acid content (C18:0) in kernels compared with non GM maize in one year, but not in the other year. The Panel considered maize NK603 to have the same composition as genetically related non GM maize lines.

In its opinion on maize MON810 under notification C/F/95/12/02, the Scientific Committee on Plants summarized the compositional analysis (SCP, 1998b). Based on the analysis of both forage and kernels of maize MON 810 and a control grown during two seasons, this committee concluded that no significant nutritional changes could be detected in maize MON 810. The Panel concurs with this previous opinion of the Scientific Committee on Plants.

##### **3.2.2. Choice of comparator and production of material for the compositional assessment**

MON 863 x MON 810 x NK603 maize was compared with the genetically modified parental lines MON 863, NK603, MON 810: a control line that has not been genetically modified (*i.e.* DKC46-26); and four commercial reference lines, also not genetically modified, in each location. The field trials were carried out in four locations in two geographically different regions in Argentina during a single season (2002-2003). In each location, three replicates were present. The choice for the four commercial reference lines could differ from one location to another and 13 commercial lines in total were employed. Argentina is one of the major exporters of maize and the locations for cultivation can be considered as being representative for regions exporting maize to the EU. Moreover, compositional data for the single events, grown during several seasons, have been described in previous applications (EFSA, 2003a,b, 2004a,b). In this case, where all parental lines have been assessed in detail by the GMO Panel or are authorised in the EU, the Panel accepts that data for comparative assessment are obtained from one growing season of MON 863 x MON 810 x NK603 maize .

For the compositional analysis of the maize plants tested, samples of whole plants and hand-pollinated ears were collected from each plot in each location. Whole plants were used for analysis of forage, while the ears were used for analysis of kernels. Plants of MON 863 x MON 810 x NK603 and NK603 maize that were sampled for compositional analysis had been treated with the herbicide glyphosate. These plants had been rendered tolerant to glyphosate by introduction of the transgenic CP4 EPSPS protein. Plants that had not been treated with glyphosate were subjected to phenotypic and agronomic analysis.

### 3.2.3. Compositional analysis

Forage was analyzed for macronutrients, calcium, and phosphorous, while kernels were analyzed for macro-nutrients, micro-nutrients, anti-nutrients, and secondary metabolites.

The compositional data thus obtained were analyzed statistically and comparisons were made between MON 863 x MON 810 x NK603 and its control, as well as between MON 863 x MON 810 x NK603 and the commercial reference lines. These comparisons were carried out for the data from each location, as well as for all data combined.

A number of statistically significant differences were observed in the comparisons of the composition of kernels from MON 863 x MON 810 x NK603 and its control. These differences, however, were not consistent throughout the various test sites. Consistent differences were observed only in oleic acid, linoleic acid, and niacin contents. For example, the levels of oleic acid increased from 29.48 % of total fatty acids in kernels of control maize to 31.73 % in those of MON 863 x MON 810 x NK603 maize. These values were within the background range of commercial varieties tested in the same experiment (22.02 - 35.24 %). The values for linoleic acid decreased from 53.06 % in control maize to 50.63 % in MON 863 x MON 810 x NK603 maize, which were also within the background range of commercial varieties (48.77 - 62.71 %). Niacin levels decreased from 20.81 mg / kg dw in control maize to 18.13 mg / kg dw in MON 863 x MON 810 x NK603 maize, while the latter was slightly outside the background range of commercial varieties (18.23 - 31.02 mg / kg dw), but still within the background ranges reported in literature (9.3 - 70 mg / kg dw) and from previous field trials with maize (14.1 - 36.3 mg / kg dw).

In addition, it is reported in literature that the fatty acid composition of maize kernels can vary substantially between maize varieties, which is influenced particularly by genetic factors (e.g. Dunlap et al., 1995). The Panel therefore considers the compositional changes in MON 863 x MON 810 x NK603 maize as small in size and not meaningful from a biological point of view. In addition, the Panel considers it unlikely that these differences would lead to adverse health effects for humans and animals.

### 3.2.4. Agronomic traits and GM phenotype

Plants of the same field trials as for compositional analysis, except for a difference in glyphosate treatment (see 3.2.2.) were compared for their agronomic and phenotypic characteristics. These characteristics included seedling vigour, crop growth stages (for example, the stage at which silking and pollination occurred), height of the plant and ear (attachment containing the cob and kernels), root lodging (plants leaning to the surface), stalk lodging (plants with stalks broken below the ear), dropped ears, final stand count, stay-green, and kernel yield. The plants tested showed no particular deviations in any of these parameters. In addition, plant damage due to insect feeding in two locations and due to weather in one location appeared to occur preferentially in plots planted with reference lines.

### 3.3. Conclusion

The MON 863 x MON 810 x NK603 maize was found to contain transgenic proteins associated with the introduced transgenic traits of insect- and herbicide-resistance (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 reference lines. The Panel therefore concludes that MON 863 x MON 810 x NK603 maize is compositionally and phenotypically equivalent to its parental single-trait GM lines and non-genetically modified maize, except for the introduced traits.

## 4. Food/feed safety assessment

### 4.1. Issues raised by Member States

(1) An extension of the testing for potential toxicity and allergenicity with additional studies, including allergenicity testing of the whole product, was recommended; (2) a post market monitoring plan for food and feed was also suggested.

### 4.2. Evaluation of relevant scientific data

#### 4.2.1. Evaluation of the single events

##### MON863

The results of 90-day sub-chronic rodent studies do not indicate adverse effects from consumption of maize line MON 863 and the Panel concluded that there are no resultant concerns over their safety. The dossier contains well-performed toxicological studies with the relevant species of animals and a statistically well-designed set-up. The Panel concluded that there are valid scientific arguments that the data provided for MON 863 supports its safety evaluation. An allergy risk evaluation of the Cry3Bb1 protein was completed, providing indirect evidence for a low probability of allergenicity. The allergenicity of the whole crop might be increased as an unintended effect, but this issue does not appear relevant to the Panel since maize is not considered a common allergenic food.

MON 863, has been studied in nutritional feeding studies with broilers and showed no adverse effects. The Panel considered that the nutritional properties of maize MON 863 would be no different from those of conventional maize (EFSA, 2004a,b and references therein) .

##### NK603

As a result of the genetic modification NK603 contains two slightly different CP4 EPSPS proteins expressed from two copies of the *cp4 epsps* gene using different promoters. The proteins differ from each other in one amino acid. Analysis of the impact of this change indicated no apparent changes in EPSPS protein structure, activity, toxicity or allergenicity using appropriate bioinformatics 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 was as safe as its non-GM comparator. Analysis of the grain from field trials in the USA and Europe showed that NK603 had the same composition as its non-GM comparator (EFSA, 2003a,b).

## 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 maize line MON 810, and therefore there are no resultant concerns over its safety. For this single insert line, the dossier contains well-performed toxicological studies with the relevant species of animals and a statistically well-designed set-up. An allergy risk evaluation of the Cry1Ab was completed, providing indirect evidence for a low probability of allergenicity. The allergenicity of the whole crop does not appear relevant to the Panel since maize is not considered a common allergenic food.

MON 810, maize have been studied in nutritional feeding studies with broilers and showed no adverse effects. The Panel considers that the nutritional properties of maize MON 810 would be no different from those of conventional maize (SCP, 1998b, EFSA 2004a,b).

### 4.2.2. Product description and intended use

The application covers the food and feed uses, import and processing of MON 863 x MON 810 x NK603 maize, as stipulated in Regulation (EC) No 1829/2003. MON 863 x MON 810 x NK603 maize, is to be used as conventional maize for the purpose of food and feed. Conventional maize is processed, for example, to starch and germ oil, which are used as food, and protein-rich by-products, such as gluten meal and corn gluten feed, which are used as feed.

### 4.2.3. Stability during processing

Since MON 863 x MON 810 x NK603 maize has been found to be substantially equivalent to conventional maize, except for the introduced traits, considerations of the stability of any altered nutritional components do not pertain to this in the Panel's opinion.

### 4.2.4. Toxicology

#### 4.2.4.1. *Toxicological assessment of expressed novel proteins in MON863 x MON810 x NK603 maize*

The safety of the CP4 EPSPS and CP4 EPSPS L214P transgenic proteins has previously been assessed for the NK603 maize, on which the Panel previously issued its opinion (EFSA, 2003a,b). In a similar fashion, the safety of the Cry3Bb1 and NptII proteins has previously been assessed for MON863 maize by the Panel (EFSA, 2004a,b), while the safety for the Cry1Ab protein has been assessed for MON810 maize by the Scientific Committee on Plants (SCP, 1998b).

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

#### 4.2.4.2. *Toxicological assessment of new constituents other than proteins*

As summarized under the section on compositional analysis, no relevant changes have been observed in MON 863 x MON 810 x NK603 and therefore no further safety assessment of new constituents in MON 863 x MON 810 x NK603 is warranted.

#### **4.2.4.3. Toxicological assessment of the whole GM food/feed**

##### **Subchronic oral toxicity**

As stated above, the toxicological safety of the parental lines has been assessed in previous opinions (SCP 1998b; EFSA 2003a,b; EFSA 2004a,b). A 90-days oral toxicity study with MON 863 x MON 810 x NK603 maize in rats, which was submitted at a later date, has been assessed by the GMO Panel.

A 90-day oral toxicity study on Sprague Dawley rats has been carried out with diets prepared with the whole food, *i.e.* maize kernels. The experiment was carried out with three groups of 40 animals each (20 animals/sex/group). During a 90 day-period, the animals received a specific diet containing 33% (w/w) maize. The control group received a diet containing 33% near-isogenic control maize, while the two test groups received diets containing either 33% transgenic maize or 11% transgenic maize supplemented with 22% control maize. The study design followed OECD guidelines 408 (OECD, 1998).

During the study, animals were checked daily for their appearance and behavior. Body weights and feed consumption were measured weekly. Clinical pathology measurements, including serum chemistry, urine chemistry, and hematology, were carried out at the termination of the experiment. A number of isolated tissues, organs, and lesions (if present) from the animals fed 33% control maize or 33% transgenic maize were further inspected microscopically.

A limited number of differences between the test groups and the controls were observed. The results of blood cell analysis showed that the average absolute counts of neutrophils in females fed 11% transgenic maize were higher than those in animals fed control maize, whereas those in animals fed the 33% test maize were not significantly changed. However, there was no difference between test and control groups in relative counts of neutrophils compared with total white blood cells. In addition, the ranges of the control, 11% test and 33% test groups overlapped with each other. The Panel therefore considers this finding neither as dose-related nor as toxicologically relevant.

In addition, the volume of urine was lower in males in both test groups as compared with that in the control group. Three out of ten animals of the male control group had high urine volumes. No other features that might have been linked to this observation were observed. In addition, no aberrations were observed in the female control group. The Panel therefore considers this finding as not toxicologically relevant.

Higher absolute weights of the epididymes were observed in males of the test group fed a diet containing 11% transgenic maize as compared with the corresponding weights in the control group. The average value for the 33% test group, however, was not different from the control. In addition, there were no differences between the various groups if the epididymes weights were expressed relative to body weight or brain weight. Therefore the Panel considers the increased epididymes weight in the 11% test group neither as dose-related nor as giving rise to safety concerns.

##### **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 the transformation may have altered the allergenic properties of the modified food. A weight-of-evidence approach is

recommended, taking into account all of the information obtained with various test methods, since no single experimental method yields decisive evidence for allergenicity (EFSA, 2004c; CAC, 2003).

#### **4.2.5.1. Assessment of allergenicity of the newly expressed proteins**

The potential allergenicity of the transgenic proteins expressed in MON 863 x MON 810 x NK603 maize has previously been assessed by the Panel in the frame of the application procedures for the parental lines MON 863, MON 810, and NK603. No new data have come to light that would suggest that the characteristics of the transgenic proteins expressed in MON 863 x MON 810 x NK603 maize would have been altered as compared with its counterparts in MON 863, MON 810, and NK603. In kernels of maize MON863 x MON810 x NK603, transgenic proteins Cry1Ab, Cry3Bb1, and CP4 EPSPS were detected at levels similar to those of Cry1Ab in MON 810, Cry3Bb1 in MON 863, and CP4 EPSPS in NK603 (section 2.2.4.).

An allergy risk evaluation of Cry1Ab and Cry3Bb1 proteins has been completed using different approaches. This led to indirect evidence for an allergenicity risk for either protein being very low. Evidence included the absence of known allergenicity of the source, absence of sequence homology with known allergens and rapid and extensive degradation by pepsin (Metcalf *et al.*, 1996, EFSA, 2004c; CAC, 2003). Previous applications of Cry1Ab using the same strategy were evaluated by national competent authorities and the EC Scientific Committees and EFSA (SCP, 1998a,b,c; SCP, 2000; SCF, 2002; EFSA 2005) and approved (EC, 1998a,b,c; EC, 1999; EC, 2004c). The Panel is not aware of any new information on allergenicity that requires a change in this opinion. Also, the Panel is not aware of any new, validated tests that produce more relevant or accurate information on possible allergenicity of the protein and that would provide a higher guarantee of safety.

Cry3Bb1 protein from *E. coli* and from MON 863 maize was digested to a low molecular fragment under standardised simulated gastric fluid. The low molecular fragment (~3 kDa) was further digested to below the limit of detection. These results show that the MON 863 Cry3Bb1 protein is not stable to digestion in simulated gastric fluid and therefore it is unlikely that fragments would elicit an allergic response.

A European country mentioned literature about 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.*, 1999; 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).

In the same manner, Cry1Ac 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.*, 1999; Moreno-Fierros *et al.*, 2003). The Panel is of the opinion that as maize is not a common allergenic food, and only a rare cause of occupational allergy, the adjuvant effect of Cry proteins, observed after high dosage intragastric or intranasal administration will not raise any concerns regarding allergenicity.

#### **4.2.5.2. Assessment of allergenicity of the whole GM plant**

Another issue is that 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 (*i.e.* in MON 863, MON 810, and NK603) but also in the triple transgenic plant after crossbreeding of MON 863, MON 810, and NK603. However, this issue does not appear relevant to the 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 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

The nutritional characteristics of MON 863 x MON 810 x NK603 maize have been tested as diet ingredient for chicken broilers. Given their rapid growth, *i.e.* full size within approximately six weeks, these animals are considered to be a sensitive model to test for any unforeseen nutritional changes within the maize lines under consideration.

Diets containing 55-60 % maize kernels of a specific line were fed to chicken broilers. The maize lines tested were MON 863 x MON 810 x NK603, a control line that has not been genetically modified (*i.e.* DKC46-26), and five non-transgenic commercial reference lines, which had been grown in Hawaii. Each diet was fed to a group of 100 animals divided into separate pens.

Animal performance was analyzed by measuring the body weight, feed intake, and mortality. After termination, the *post mortem* weights of the carcass and edible parts were analyzed, as well as the composition of the edible parts.

Statistically significant differences occurred in the weights of edible parts, *i.e.* breast meat and wings, between animals fed MON 863 x MON 810 x NK603 and control maize. The average absolute breast meat weight was 0.433 kg in animals fed MON 863 x MON 810 x NK603 and 0.455 kg in the controls. The relative weight of wings was 11.857 % of chill weight in the MON 863 x MON 810 x NK603 group and 11.637 % in the control group. These differences are of comparatively slight magnitude and were not observed in the comparison between the test group fed MON 863 x MON 810 x NK603 and several groups of animals fed the reference lines.

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

MON 863 x MON 810 x NK603 maize is, from a nutritional point of view, equivalent to conventional maize and will be used as any other maize. The GMO Panel is of the opinion that a post-market monitoring of the GM food/feed is not regarded necessary.

### 4.3. Conclusion

Evidence has been provided in previous evaluations that there is no acute toxicity from the CP4 EPSPS, Cry3Bb1, Cry1Ab and NptII proteins.

The Panel considers that the data from the 90 days rat feeding study with grain from MON 863 x MON 810 x NK603 maize are sufficient to conclude that there is no reason to assume that the MON 863 x MON 810 x NK603 have any other influence on human and animal health than

conventional maize. Moreover this study confirms the absence of adverse effects of the combination of the expressed proteins.

An allergy risk evaluation of the Cry1Ab, Cry3Bb1 and CP4 EPSPS proteins was completed, providing indirect evidence for a low probability of allergenicity. The allergenicity of the whole crop does not appear to be relevant to the Panel since maize is not considered a common allergenic food.

MON 863 x MON 810 x NK603 maize has been studied in nutritional feeding studies with broilers and showed no adverse effects. The Panel concludes that the broiler study was adequate to establish nutritional equivalence and considers that the nutritional properties of maize MON 863 x MON 810 x NK603 would be no different from those of conventional maize.

## **5. Environmental risk assessment and monitoring plan**

### **5.1. Issues raised by Member States**

Comments from Member States included the following: (1) A need to address the impacts of unintended release and the effects of Cry proteins on non-target species; (2) a need to address the consequence of water and soil exposure to the toxins present in MON 863 x MON 810 x NK603 via organic waste material and litter or sewage, which occur during processing or through spillage; (3) a need for a more detailed post market monitoring plan including more details on general surveillance methods.

### **5.2. Evaluation of relevant scientific data**

#### **5.2.1. Evaluation of the single events**

##### **MON 863 and MON 810**

MON 863 maize has been assessed for import only (EFSA, 2004a,b) and thus there was no requirement for scientific information on environmental effects associated with cultivation. MON810 was assessed for import and cultivation (SCP, 1998b; EFSA, 2004a,b; EFSA, 2005).

The 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 flow to bacteria (see section 5.2.2.4). Data supplied by the applicant and other literature suggested that most of the protein would be degraded by enzymatic activity in the intestinal tract. Data also indicate that limited amounts of proteins that would remain intact and pass out in the faeces would subsequently be further degraded in the manure due to microbial processes. Thus amounts of intact 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 coleoptera).

There is an issue in that the *cry1Ab* gene in MON 810 is synthetic producing 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 would be the greatest 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.

### **NK603**

The approved notification C/ES/00/01 for maize NK603 only concerned import. 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. In conclusion, the 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 or feed or processing is unlikely to have an adverse effect on human or animal health or, in that context, on the environment (EFSA, 2003a,b).

## **5.2.2. Environmental risk assessment**

### **5.2.2.1. Potential unintended effects on plant fitness due to the genetic modification**

Maize is highly domesticated and not generally able to survive in the 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 or disturbed land in Europe, despite cultivation for many years. In addition, there are no cross-compatible wild relatives in Europe, and gene flow via pollen is largely restricted to neighbouring crops.

MON 863 x MON 810 x NK603 maize has no altered survival, multiplication or dissemination characteristics except in the presence of the specific herbicide or target organisms. The Panel is of the opinion that the likelihood of unintended environmental effects due to the establishment and spread of this maize will be no different to that of MON 863, MON 810 or NK603 maize and traditionally maize varieties.

### **5.2.2.2. Potential for gene transfer**

A prerequisite for any gene transfer is the availability of pathways for the transfer of genetic material, DNA in case of horizontal gene transfer and pollen in case of vertical gene flow through cross-pollination.

Exposure of microorganisms to transgenic DNA derived from GM maize plants takes place in the environment during natural decay of transgenic plant material, such as GM plant parts, in agricultural areas and/or pollen in nearby natural ecosystems as well as in cropped fields. 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 (domesticated animals and other 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. Since MON 863 x MON 810 x NK603 maize has no altered survival, multiplication or dissemination characteristics, except in the presence of the

specific herbicide or target insects, the Panel is of the opinion that the likelihood of unintended environmental effects due to the establishment and spread of this maize will be no different to that of MON 863, MON 810 or NK603 maize and traditionally bred maize.

#### (a) Plant to bacteria gene transfer

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

The *cry3Bb1*, *nptII*, *cry1Ab* and *cp4 epsps* are under the control of eukaryotic promoters with limited if any activity in prokaryotic organisms. Genes under control of prokaryotic regulatory elements conferring the same traits as expressed in the GM plants are widespread in microorganisms in natural environments.

The specific issue of gene transfer of synthetic *cry1Ab* is discussed under section 5.2.1.

Taking into account the origin and nature of the *cry* and *cp4 epsps* genes and the lack of selective pressure in the intestinal tract and/or the environment, the likelihood that horizontal gene transfer would confer selective advantages or increased fitness on microorganisms is very limited. For this reason it is very unlikely that *cry* genes and *cp4 epsps* from MON 863 x MON 810 x NK603 maize would become established in the genome of microorganisms in the environment or human and animal digestive tract. In the very unlikely event that such a horizontal gene transfer would take place, no adverse effects on human and animal health and the environment are expected as no principally new traits would be introduced into microbial communities.

Maize line MON 863 contains an intact *nptII* gene encoding neomycin phosphotransferase II. This gene was used as a selection marker during the construction of event MON 863 and is retained in the MON863 x MON810 x NK603 maize. The EFSA GMO Panel recently formulated an Opinion (EFSA, 2004d) on the use of antibiotic resistance genes in GM plants and concluded that the use of *nptII* as a selection marker did not pose a risk to the environment or to human and animal health. This conclusion was based on the limited use of kanamycin and neomycin in human and veterinary medicine, the already widespread presence of this gene in bacterial populations and the low risk of trans-kingdom gene transfer from plants to bacteria (reviewed by Bennett *et al.*, 2004). The *nptII* gene is a well-established selection marker with a history of safe use (Nap *et al.*, 1992; Redenbaugh *et al.*, 1994). This conclusion is consistent with earlier safety evaluations of *nptII* (SCP, 1998a).

#### (b) Plant to plant gene transfer

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. The extent of cross-pollination to conventionally bred hybrids will mainly depend on the scale of accidental release and/or adventitious presence in conventional seeds. 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.

As shown in several field trials there are no indications for an altered ecological fitness of the GM maize in comparison to conventionally bred hybrids with similar genetic background.

Insect protection against lepidopteran and coleopteran pests is also not regarded as providing a selective advantage for maize in Europe, as the survivability is mainly limited by the absence of a dormancy phase, susceptibility to fungi and susceptibility to cold climate conditions. Therefore, as

for any other maize cultivars, volunteers would only survive until subsequent seasons in the warmer regions of Europe and are not likely to establish feral or undesirable populations under European environmental conditions.

Studies in Europe and elsewhere with NK603 maize have not shown any enhanced weediness or fitness, except in the presence of the specific herbicide.

Since enhanced survival, multiplication or dissemination characteristics are only likely when MON 863 x MON 810 x NK603 maize is cultivated in the presence of the specific herbicide or target insects, the Panel is of the opinion that the likelihood of unintended environmental effects due to the establishment and spread of this maize will be no different to that of MON 863, MON 810 or NK603 maize and traditionally bred maize.

### **5.2.2.3. Potential interactions of the GM plant with non-target organisms**

There is an issue that gene products, particularly Cry proteins might enter the environment either from the gastrointestinal tracts of animals (manure and faeces), through horizontal gene flow to bacteria or as part of waste waters and/or dusts from food and feed production. Data supplied by the applicant and other literature (Ahmad et al., 2005; and references therein) suggests that most proteins would be degraded in the environment. In addition enzymatic activity in the gastrointestinal tract would degrade Cry toxin so that little would remain intact 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, minimising the possibility for exposure of potentially sensitive non-target organisms.

### **5.2.3. 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 and identified no potential adverse environmental impacts. Since the main use of MON 863 x MON 810 x NK603 maize will be animal feeds, the applicant proposed that general surveillance should concentrate on monitoring the health of those exposed to the processing of the animal feed as well as the animals fed on this maize. The Panel agrees to this proposed generic approach to general surveillance.

### **5.3. Conclusion**

MON 863 x MON 810 x NK603 maize is being assessed for import 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 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 scope of the monitoring plan provided by the applicant is in line with the intended uses for the GMO since this does not include cultivation. The Panel advises that appropriate management systems should be in place to restrict seeds of GM 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 maize MON 863 x MON 810 x NK603 which is produced by a cross between inbred lines of maize containing MON 863, MON 810 and NK603 events. The MON 863, MON 810 and NK603 maize were evaluated previously (EFSA, 2004a,b; SCP, 1998b; EFSA, 2003a,b) and MON 810 and NK603 has been authorized (EC, 1998a,b, 2004a; 2005). In assessing the MON 863 x MON 810 x NK603 maize, both the single insert lines and MON 863 x MON 810 x NK603 maize were considered. The Panel concluded that it was acceptable to use data for the single insert lines MON 863, MON 810 and NK603 in support of the safety assessment of the MON 863 x MON 810 x NK603 maize.

The results of the safety assessment do not indicate adverse effects from consumption of MON 863 x MON 810 x NK603 maize and the Panel concludes that there are no concerns over its safety.

In conclusion, the Panel considers that the information available for MON 863 x MON 810 x NK603 maize addresses the outstanding questions raised by the Member States and considers that it will not have an adverse effect on human and animal health or the environment in the context of its proposed use.

## DOCUMENTATION PROVIDED TO EFSA

1. Letter from the Belgian Competent Authority (SPF Santé publique, Sécurité de la Chaîne alimentaire et Environnement), dated 23 November 2004 concerning the submission to EFSA of application MON 863 x MON 810 x NK603 maize within the framework of Regulation (EC) No 1829/2003 .
2. Letter from EFSA to applicant, dated 14 January 2005, concerning the "Statement of Validity" for application EFSA-GMO-BE-2004-07 on MON 863 x MON 810 x NK603 maize submitted under Regulation (EC) No 1829/2003 (Ref. SR/KL/jq (2005) 066).
3. Submission of the application EFSA-GMO-BE-2004-07 by the applicant to EFSA, containing:
  - Part I - technical dossier
  - Part II - summary
  - Part III - Cartagena Protocol
  - Part IV - labelling proposal
  - Part V - samples and detection method
  - Part VI - additional information for GMOs
4. Letter from EFSA to applicant, dated 9 February 2005, to stop the clock on behalf of JRC-CRL for application EFSA-GMO-BE-2004-07 on MON 863 x MON 810 x NK603 maize submitted under Regulation (EC) No 1829/2003 (Ref. SR/MR/jq/ (2005) 163).
5. Letter from EFSA to applicant, dated 27 April 2005 requesting access to a 90 day rat study with MON 863 x MON 810 x NK603 maize (Ref. SR/AC/jq/ (2005) 473).
6. Letter from applicant to EFSA, dated 19 May 2005, providing a 90 day feeding study in rats in the context of application EFSA-GMO-BE-2004-07 on MON 863 x MON 810 x NK603 maize submitted under Regulation (EC) No 1829/2003 .

7. Comments of the Member States, including the national Competent Authorities within the meaning of Directive 2001/18/EC following the requirements of Article 6(4) and 18(4) of Regulation (EC) No 1829/2003 (GMO EFSAnet).

## REFERENCES

- ACNFP, 1996. ACNFP Report on Processed Products from GM Insect-Resistant Maize (Annex IV). In: ACNFP Annual Report 1996. Advisory Committee on Novel Foods and Processes, London, pp. 55-64.  
[http://www.food.gov.uk/multimedia/pdfs/acnfp\\_app\\_i-vi.pdf](http://www.food.gov.uk/multimedia/pdfs/acnfp_app_i-vi.pdf)
- Ahmad, A., Wilde G.E., Zhu K.Y., 2005. Detectability of Coleopteran-specific Cry3Bb1 protein in soil and its effect on nontarget surface and below-ground arthropods. *Environ. Entomol.*, 34, 385-394
- Bennett, P. M., Livesey, C. T., Nathwani, D., Reeves, D. S., Saunders, J. R. and Wise, R., 2004. An assessment of the risks associated with the use of antibiotic resistance genes in genetically modified plants: report of the Working Party of the British Society for Antimicrobial Chemotherapy. *J. Antimicrob. Chemother.*, 53, 418-431.  
<http://jac.oupjournals.org/cgi/reprint/53/3/418.pdf>
- Bjorklund 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(1);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>
- Crickmore, N., Zeigler, D.R., Feitelson, J., Schnepf, E., Van Rie, J., Lereclus, D., Baum, J. and Dean, D.H., 1998. Revision of the nomenclature for the *Bacillus thuringiensis* pesticidal crystal proteins. *Microbiol. Mol. Biol. Rev.* 62, 807-813.
- Dunlap, F.G., White, P.J., Pollak, L.M., 1995. Fatty acid composition of oil from exotic corn breeding materials. *Journal of the American Oil Chemists' Society* 72, 989-993.
- EC, 1990. Council Directive 90/220/EEC of 23 April 1990 on the deliberate release into the environment of genetically modified organisms. *OJ L117*, 15-27.  
[http://europa.eu.int/smartapi/cgi/sga\\_doc?smartapi!celexapi!prod!CELEXnumdoc&lg=EN&numdoc=31990L0220&model=guichett](http://europa.eu.int/smartapi/cgi/sga_doc?smartapi!celexapi!prod!CELEXnumdoc&lg=EN&numdoc=31990L0220&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](http://europa.eu.int/smartapi/cgi/sga_doc?smartapi!celexapi!prod!CELEXnumdoc&lg=EN&numdoc=31997R0258&model=guichett)
- EC, 1998a. Commission Decision of 22 April 1998 concerning the placing on the market of genetically modified maize (*Zea mays* L. line MON 810), 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](http://europa.eu.int/eur-lex/pri/en/oj/dat/1998/l_131/l_13119980505en00320033.pdf)

- EC, 1998b. Summary of notifications received in 1997 by the Commission pursuant to Article 5 of European Parliament and Council Regulation (EC) No 258/97. Official Journal of the European Communities C 200, 16.  
[http://europa.eu.int/eur-lex/pri/en/oj/dat/1998/c\\_200/c\\_20019980626en00160016.pdf](http://europa.eu.int/eur-lex/pri/en/oj/dat/1998/c_200/c_20019980626en00160016.pdf)
- EC, 1998c. Commission Decision of 22 April 1998 concerning the placing on the market of genetically modified maize (*Zea mays* L. line Bt-11), pursuant to Council Directive 90/220/EEC.  
[http://europa.eu.int/eur-lex/pri/en/oj/dat/1998/l\\_131/l\\_13119980505en00280029.pdf](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) No 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](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](http://europa.eu.int/eur-lex/pri/en/oj/dat/2001/l_106/l_10620010417en00010038.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](http://europa.eu.int/eur-lex/pri/en/oj/dat/2003/l_268/l_26820031018en00010023.pdf)
- EC, 2004a. 2004/643/EC: 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 (notified under document number C(2004) 2761)(Text with EEA relevance). OJ, L295, 35-37.  
[http://europa.eu.int/eur-lex/pri/en/oj/dat/2004/l\\_295/l\\_29520040918en00350037.pdf](http://europa.eu.int/eur-lex/pri/en/oj/dat/2004/l_295/l_29520040918en00350037.pdf)
- EC, 2004c. Commission Decision of 19 May 2004 authorising the placing on the market of sweet corn from genetically modified maize line Bt11 as a novel food or novel food ingredient under Regulation (EC) No 258/97 of the European Parliament and of the Council (notified under document number C(2004) 1865). OJ, L300, 48-51.  
[http://europa.eu.int/eur-lex/lex/LexUriServ/site/en/oj/2004/l\\_300/l\\_30020040925en00480051.pdf](http://europa.eu.int/eur-lex/lex/LexUriServ/site/en/oj/2004/l_300/l_30020040925en00480051.pdf)
- EC, 2005. 2005/448/EC: Commission Decision of 3 March 2005 authorising the placing on the market of foods and food ingredients derived from genetically modified maize line NK 603 as novel foods or novel food ingredients under Regulation (EC) No 258/97 of the European Parliament and of the Council (notified under document number C (2005) 580). OJ, L158, 20-22.  
[http://europa.eu.int/eur-lex/lex/LexUriServ/site/en/oj/2005/l\\_158/l\\_15820050621en00200022.pdf](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 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 (Question No EFSA-Q-2003-003). EFSA Journal 10, 1-13  
[http://www.efsa.eu.int/science/gmo/gmo\\_opinions/176/opinion\\_gmo\\_03\\_final\\_en1.pdf](http://www.efsa.eu.int/science/gmo/gmo_opinions/176/opinion_gmo_03_final_en1.pdf)
- EFSA, 2003b. 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](http://www.efsa.eu.int/science/gmo/gmo_opinions/177/opinion_gmo_02_final_en1.pdf)
- EFSA, 2004a. 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 MON 810, for import and processing, under Part C of Directive 2001/18/EC from Monsanto (Question No EFSA-Q-2003-089). EFSA Journal 49, 1-25.  
[http://www.efsa.eu.int/science/gmo/gmo\\_opinions/381/opinion\\_gmo\\_06\\_en1.pdf](http://www.efsa.eu.int/science/gmo/gmo_opinions/381/opinion_gmo_06_en1.pdf)
- EFSA, 2004b. 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 MON 810, 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-2003-121). EFSA Journal 50, 1-25.  
[http://www.efsa.eu.int/science/gmo/gmo\\_opinions/383/opinion\\_gmo\\_07\\_en1.pdf](http://www.efsa.eu.int/science/gmo/gmo_opinions/383/opinion_gmo_07_en1.pdf)
- EFSA, 2004c. 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](http://www.efsa.eu.int/science/gmo/gmo_guidance/660_en.html)
- EFSA, 2004d. Opinion of the Scientific Panel on Genetically Modified Organisms on the use of antibiotic resistance genes as marker genes in genetically modified plants. EFSA Journal 48, 1-18  
[http://www.efsa.eu.int/science/gmo/gmo\\_opinions/384\\_en.html](http://www.efsa.eu.int/science/gmo/gmo_opinions/384_en.html)
- EFSA, 2005. Opinion of the Scientific Panel on Genetically Modified Organisms on a request from the Commission related to the notification (Reference C/F/96/05.10) for the placing on the market of insect-tolerant genetically modified maize Bt11, for cultivation, feed and industrial processing, under Part C of Directive 2001/18/EC from Syngenta Seeds. (Question No EFSA-Q-2004-012) EFSA Journal (2005) 213, 1-33.  
[http://www.efsa.eu.int/science/gmo/gmo\\_opinions/922/gmo\\_opinion\\_ej213\\_bt11maize\\_cultivation\\_en1.pdf](http://www.efsa.eu.int/science/gmo/gmo_opinions/922/gmo_opinion_ej213_bt11maize_cultivation_en1.pdf)
- Metcalf, 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, Ruiz-Medina, E.J., Esquivel, R.L., Lopez-Revilla, R., Pina-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.
- Nap, J.P., Bijvoet, J. & Stiekema, W.J., 1992. Biosafety of kanamycin-resistant transgenic plants: an overview. Transgenic Res., 1, 239-249.

- OECD, 1998. Test Guideline 408. Repeated Dose 90-day Oral Toxicity Study in Rodents. In: OECD Guidelines for the testing of chemicals. Organization for Economic Cooperation & Development, Paris.
- Redenbaugh, K., Hiatt, W., Martineau, B., Lindemann, J. and Emlay, D., 1994. Aminoglycoside 3'-phosphotransferase II: review of its safety and use in the production of genetically engineered plants. *Food Biotech.*, 8, 137-165.
- SCF, 2002. Opinion on a request to place genetically modified sweet maize line Bt11 on the market (expressed on 17 April 2002).  
[http://europe.eu.int/comm/food/fs/sc/scf/out129\\_en.pdf](http://europe.eu.int/comm/food/fs/sc/scf/out129_en.pdf)
- SCP, 1998a. 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](http://europa.eu.int/comm/food/fs/sc/scp/out10_en.html)
- SCP, 1998b. 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](http://europa.eu.int/comm/food/fs/sc/scp/out02_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](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](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. *Int. Arch. Allergy Immunol.*, 133, 101-112.
- Vazquez-Padron R.I., Moreno-Fierros L., Neri-Bazan L., de la Riva G.A. and 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 R.I., Moreno-Fierros L., Neri-Bazan L., de la Riva G.A. and 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. and 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, 147-155.

## **SCIENTIFIC PANEL MEMBERS**

Hans Christer Andersson, Detlef Bartsch, Hans-Joerg Buhk, Howard Davies, Marc De Loose, Michael Gasson, Niels Hendriksen, John Heritage, Sirpa Kärenlampi, Ilona Kryspin-Sørensen, Harry Kuiper, Marco Nuti, Fergal O'Gara, Pere Puigdomenech, George Sakellaris, Joachim Schiemann, Willem Seinen, Angela Sessitsch, Jeremy Sweet, Jan Dirk van Elsas and Jean-Michel Wal.

## **ACKNOWLEDGEMENT**

The GMO Panel wishes to thank Gijs Kleter and Richard Phipps for their contributions to the draft opinion.

---