

Opinion of the Scientific Panel on Genetically Modified Organisms on an application (Reference EFSA-GMO-UK-2004-06) for the placing on the market of insect-protected glyphosate-tolerant genetically modified maize MON863 x NK603, for food and feed uses, and import and processing under Regulation (EC) No 1829/2003 from Monsanto¹
(Question No EFSA-Q-2004-154)

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 NK603 (Unique Identifier MON-00863-5 x MON-00603-6) to provide protection against specific 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 MON 863 and NK603 on the market under EU regulatory procedures was taken into account where appropriate.

MON 863 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. MON863 has received an EFSA opinion in favour of its authorisation. NK603 was developed to be tolerant to the herbicide glyphosate by the introduction of a gene coding for 5-enoylpyruvylshikimate-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 and food ingredients from NK603 maize was authorised under Regulation (EC) No 258/97 by Commission Decision 2005/448/EC.

MON863 x NK603 maize was produced by crosses between maize inbred lines containing MON 863 and NK603 events to combine the rootworm resistance trait in MON 863 and the tolerance to the herbicide glyphosate in NK603.

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

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

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

MON863 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 MON863 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 NK603 maize indicated that there are no adverse effects from its consumption.

Feeding studies conducted on broilers with MON 863 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-UK-2004-06 concerns food and feed uses, 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 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 863, NK603, MON 863 x NK603, insect protection, MON-00863-5 x MON-00603-6, glyphosate resistance, Cry3Bb1, NptII, CP4 EPSPS, food safety, feed safety, human health, environment, import, Regulation (EC) No 258/97, Directive 2001/18/EC, Regulation (EC) No 1829/2003.

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BACKGROUND

On 10 November 2004 EFSA received from the UK Competent Authority an application (Reference EFSA-GMO-UK-2004-06), for authorisation of MON863 x NK603 maize (Unique Identifier MON-00863-5 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-UK-2004-06 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 website². 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 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 20 June 2005. After receipt of the full data package, the GMO Panel finalised its risk assessment of MON863 x NK603 maize.

The Scientific Panel on Genetically Modified Organisms carried out a scientific assessment of the genetically modified MON863 x NK603 maize 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.

The single events MON 863 and NK603 have been the subjects of earlier assessments and have received an EFSA opinion in favour of their authorisation (EFSA, 2004a,b; 2003a,b). NK603 was

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

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 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.

ASSESSMENT

1. Introduction

The GM maize MON 863 x NK603 is assessed with reference to its intended uses 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 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 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 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 the MON 863 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 the MON 863 x NK603 maize; (2) comments were made regarding the differences in expression of the Cry proteins in the MON 863 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

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. Traditional breeding methods were used to produce MON 863 x NK603 maize and no new genetic modification was involved. The two inserts that are present in MON 863 x NK603 were derived from maize lines containing two independent events: MON 863 and NK603. Each of these GM maize events was the subject of an earlier safety evaluation and separate Opinions (EFSA, 2004a,b; EFSA, 2003a,b) for each of them have been published. MON 863 x NK603 combines the insect protection traits from MON 863 with the glyphosate resistance in NK603.

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).

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

A cross between the two transgenic lines was used to construct the maize MON 863 x NK603. The molecular structures of the DNA inserts present in this maize were investigated using Southern analyses. This involved the use of DNA probes for the *cry3Bb1* gene present in MON 836 and the *cp4 epsps* gene present in NK603. When genomic DNA digested with *EcoRV* was hybridised with the *cry3Bb1* probe, the expected 10kb MON863-specific DNA fragment was detected. This fragment includes part of the inserted DNA and one of the flanking regions. When genomic DNA digested with *EcoRV* was hybridised with the *cp4 epsps* probe, the expected 2.8kb and 3.8kb NK603-specific DNA fragments were detected. A fragment size inconsistency in the dossier was resolved by reference to the insert DNA sequence for event NK603. These fragments represent the majority of the inserted DNA. The fingerprints detected were consistent with the combination of the MON 863 and NK603 inserts in this maize. This additional analysis confirmed that both insert structures were retained in this maize. The 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 insert

The expression levels of the Cry3Bb1, CP4 EPSPS, and NptII proteins were measured by an immunochemical assay. Cry3Bb1 and CP4 EPSPS, but not NptII, occurred at detectable levels in kernels. The levels of Cry3Bb1 (average plus minus standard deviation) in kernels were 34 ± 5.4 $\mu\text{g/g dw}$ (dry weight) in MON 863 x NK603 and 29 ± 3.9 $\mu\text{g/g dw}$ in MON863. Those of CP4 EPSPS were 11 ± 1.5 $\mu\text{g/g dw}$ in MON863 x NK603 and 12 ± 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 NK603 are comparable with those in MON 863 and NK603.

2.2.5. Inheritance and stability of inserted DNA

The genetic stability of the inserted DNA in event MON 863 and NK603 was demonstrated. In MON 863 x NK603 the two inserts are combined. The Southern data presented show that both 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. 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 MON863 x NK603 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 levels of expressed transgenic proteins in the MON 863 x NK603 maize and the parental GM maize *i.e.* Cry3Bb1 in MON 863 x NK603 and MON 863, as well as CP4 EPSPS in MON 863 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 NK603 are comparable with those in MON 863 and NK603. In most samples, the NptII transgenic protein was undetectable in kernels of maize MON 863 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 MON 863, 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 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 to 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.

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

MON 863 x NK603 maize was compared with the genetically modified parental lines MON 863 and NK603; 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 both 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 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 maize MON 863 x NK603, and NK603 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 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 NK603 and its control. These differences, however, were not consistent throughout the various test sites. The only consistent difference occurred in arachidic acid, which increased from 0.42 % of total fatty acid concentration in control maize to 0.45 % in MON863 x NK603 maize. The ranges of arachidic acid contents, however, overlapped between MON 863 x NK603 (0.42 - 0.49 %) and control (0.38 - 0.46 %). In addition, these values fell within the background variation of the commercial reference lines used in the same experiment (0.38 - 0.54 %). For comparison, the range reported in literature is 0.1-2 %, while the historical range from previous field trials is 0.31-0.69%. 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 observed consistent differences in arachidic acid content as small in size and not meaningful from a biological point of view. In addition, the Panel considers it unlikely that this difference 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 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 MON863 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 support its safety evaluation. An allergy risk evaluation of the Cry3Bb1 proteins 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).

4.2.2. Product description and intended use

The application covers the food and feed uses of MON863 x NK603 maize, as stipulated in Regulation (EC) No 1829/2003. MON863 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 MON863 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 MON 863 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 MON 863 maize by the Panel (EFSA, 2004a,b).

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 NK603 and therefore no further safety assessment of new constituents in MON 863 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 of the Panel (EFSA 2003ab, 2004ab). A 90-days oral toxicity study with MON863 x NK603 maize, which was submitted at a later date, in rats has been assessed by the GMO Panel.

A 90-day oral toxicity study with whole maize kernels was performed using Sprague-Dawley rats divided into three groups consisting of 20 male and 20 female rats per group. One group received a diet containing 11% (w/w) MON 863 x NK603 maize kernels supplemented with 22% (w/w) of maize DKC46-26, which was not genetically modified and had a genetic background comparable to the test maize. The second group received a diet containing 33% (w/w) MON 863 x NK603 maize kernels. The third group, which served as the control group, received a diet containing 33% (w/w) kernels of maize DKC46-26. The study design followed OECD guidelines 408 (OECD, 1998).

All animals were examined daily for appearance, moribundity, and mortality. In addition, they were examined weekly for additional physical aspects. Individual body weights and food consumption were also recorded weekly. At the end of the experiment, an extensive clinical pathological evaluation was performed, including haematology, serum chemistry, and urine analysis. In addition, a complete necropsy was carried out, including both macroscopic examinations and histopathology.

With regard to feed consumption, the average daily intake per animal was statistically significantly higher during various weeks in the male and female 33% test groups. However, the ranges of individual values overlapped with each other. The Panel considers the observed differences as minor and therefore, these differences do not give rise to safety concerns.

There were no statistically significant differences in organ weights except for a lower mean heart weight and heart/brain ratio in male rats fed 11% MON 863 x NK603 compared with the controls. There was no difference in heart weight relative to body weight in this group. In addition, these differences were not observed in male rats fed 33% test maize. Therefore, the Panel is of the opinion that these differences in heart weight are not related to the test maize.

With regard to haematology parameters, the only observed statistically significant differences were lower values for mean red blood cell count and higher values for mean corpuscular hemoglobin in male rats fed 11% MON 863 x NK603 compared with the control group. However, no difference was observed in the values for mean corpuscular hemoglobin concentrations. The Panel considers the observed differences in haematology as not being related to the test article, since these differences remained unconfirmed for rats fed 33% transgenic maize.

With regard to serum chemistry, the value for mean blood urea nitrogen was statistically significantly higher in female rats in the 11% test group than in the control group. As this difference was not observed in the female 33% test group, the Panel considers this observation as unrelated to the administration of test maize to the animals.

Urinalysis parameters were not statistically significantly different when the control and test groups were compared.

Macroscopic and microscopic examinations showed no changes related to feeding rats with the test diets containing MON 863 x NK603 maize as compared to feeding the control diet.

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

An allergy risk evaluation of the Cry3Bb1 protein has been completed using different approaches. This led to indirect evidence for an allergenicity risk for this 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).

It was observed that one of these transgenic proteins, *i.e.* Cry3Bb1 protein from *E. coli* and from MON 863 maize, was digested to a low molecular fragment in standardised simulated gastric fluid containing pepsin. The low molecular fragment (~3 kDa) was further digested to below the limit of detection. The Panel concludes that 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 or crop

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 and in NK603) but also in the double transgenic plant after crossbreeding of MON 863 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 MON863 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 NK603, a non-genetically modified control line (*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 breast meat, thighs, and drums.

No consistent statistically significant effect was thus observed in these parameters between the animals fed maize MON 863 x NK603 and the control & reference lines.

4.2.7. Post-market monitoring of GM food/feed

MON 863 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, and NptII proteins.

The Panel considers that the data from the 90-days rat feeding study with grain from MON 863 x NK603 maize are sufficient to conclude that there is no reason to assume that the MON 863 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 Cry3Bb1 and CP4 EPSP 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 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 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

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.

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).

The Panel agreed that unintended environmental effects due to the adventitious establishment and spread of this GM maize will be no different to that of traditionally bred maize.

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 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 or NK603 maize and traditional 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.

(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*, 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.

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 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 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). *NptII* 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, 1998).

(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 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 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 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 protein 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 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 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 MON863 x NK603 maize which is produced by a cross between inbred lines of maize containing MON 863 and NK603 events. The MON 863 and NK603 maize were evaluated previously (EFSA, 2004a,b; 2003a,b) and NK603 has been authorized (EC, 2004a; 2005). In assessing the MON 863 x NK603 maize, both the single insert lines and the MON 863 x NK603 maize were considered. The Panel concluded that it was acceptable to use data for the single insert lines MON 863 and NK603 in support of the safety assessment of the MON 863 x NK603 maize.

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

In conclusion, the Panel considers that information available for MON 863 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 UK Competent Authority (Food Standards Agency), dated 10 November 2004 concerning the submission to EFSA of application MON 863 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-UK-2004-06 on MON 863 x NK603 maize submitted under Regulation (EC) No 1829/2003 (Ref. SR/KL/jq (2005) 065).
3. Submission of the application EFSA-GMO-UK-2004-06 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-UK-2004-06 on MON 863 x NK603 maize submitted under Regulation (EC) No 1829/2003 (Ref. SR/MR/jq/ (2005) 162).
5. Letter from EFSA to applicant, dated 27 April 2005 requesting access to a 90 day rat study with MON 863 x NK603 maize (Ref. SR/AC/jq/ (2005) 472)
6. Letter from applicant to EFSA, dated 20 June 2005, providing a 90 day feeding study in rats in the context of application EFSA-GMO-UK-2004-06 on MON 863 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).

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