

Opinion of the Scientific Panel on Genetically Modified Organisms on an application (Reference EFSA-GMO-DE-2004-03) for the placing on the market of insect-protected genetically modified maize MON 863 x MON 810, for food and feed use, under Regulation (EC) No 1829/2003 from Monsanto¹

(Question No EFSA-Q-2004-112)

Opinion adopted on 8 June 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 (Unique Identifier MON-00863-5 x MON-00810-6).

In the past, EFSA was requested to deliver its opinion on two questions raised by the Commission related to applications for the placing on the market of MON 863 x MON 810 maize under the Novel Food Regulation (EC) No 258/97 for food uses and under the Directive 2001/18/EC on the deliberate release of genetically modified organisms (GMOs) into the environment for feed uses, import and processing.

In its opinions of 2 April 2004, the Panel concluded that it was acceptable to use data for the single insert lines MON 863 and MON 810 in support of the safety assessment of MON 863 x MON 810 maize, in addition to the data package provided for MON 863 x MON 810 maize. However, the Panel was divided over the need for confirmatory data for the risk assessment of MON 863 x MON 810 maize, in particular the need for an additional 90-day rat study with MON 863 x MON 810 maize. Therefore, the Panel could not reach agreement on the safety evaluation of MON 863 x MON 810 maize. To resolve this issue, EFSA decided to request the study in question from the applicant in order to allow the Panel to finalise its evaluation.

After receipt of the full data package on the 90 days rat study, EFSA referred the issue back to the GMO Panel and asked the Panel to conclude the risk assessment of MON 863 x MON 810 maize.

Due to changes in the EU legislation, the applicant had to introduce an application under Regulation (EC) No 1829/2003 to replace the application under Regulation (EC) No 258/97 and decided to include in the scope both food and feed uses (reference EFSA-GMO-DE-2004-03). Import and processing are still covered by the scope of the notification under Directive 2001/18 (reference C/DE/02/9).

In delivering the present opinion the Panel considered the different applications regarding MON 863 x MON 810 maize, the information concerning the single insert lines MON 863 and MON 810, the additional information provided by the applicant and the specific questions and concerns, raised by the Member States. Although an overall single risk assessment has been

¹ For citation purposes: Opinion of the Scientific Panel on Genetically Modified Organisms on an application (Reference EFSA-GMO-DE-2004-03) for the placing on the market of insect-protected genetically modified maize MON 863 x MON 810, for food and feed use, under Regulation (EC) No 1829/2003 from Monsanto, The EFSA Journal (2005) 252, 1-23.

made, for regulatory reasons, opinions for the application under Regulation (EC) No 1829/2003 and the notification under Directive 2001/18/EC are issued separately.

MON 863 x MON 810 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 and food ingredients from MON 810 maize was notified in 1997 under Regulation (EC) No 258/97.

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

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

Cry3Bb1 and Cry1Ab protein levels in kernels of MON 863 x MON 810 maize were higher than in the individual MON 863 and MON 810 lines. However, the ranges were broad and there were overlaps between the levels of Cry proteins expressed in the MON 863 and MON 810 parents and in MON 863 x MON 810 maize. The Panel concludes that these data do not raise safety concerns.

The safety and the allergenic risk of the Cry3Bb1, Cry1Ab and NptII proteins have previously been assessed in the single events for which positive opinions were issued.

Feeding studies conducted on broilers with MON 863 x MON 810 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 results of the 90-day sub-chronic rodent study do not indicate adverse effects from consumption of MON 863 x MON 810 maize and the Panel concludes that there are no concerns over its safety.

The application EFSA-GMO-DE-2004-03 concerns food and feed uses. 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 Panel concludes that the amounts of Cry toxin being distributed in the environment would be very low, minimizing the possibility for exposure of potentially sensitive non-target organisms. The monitoring plan provided by the applicant is in line with the intended uses for the GMO.

In conclusion, the Panel considers that the information available for MON 863 x MON 810 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, MON 863 x MON 810, MON-00863-5 x MON-00810-6, insect protection, Cry3Bb1, Cry1Ab, NptII, food safety, feed safety, human health, environment, import, Regulation (EC) No 258/97, Regulation (EC) No 1829/2003, Directive 2001/18/EC.

TABLE OF CONTENTS

SUMMARY.....	1
BACKGROUND.....	4
TERMS OF REFERENCE.....	6
ASSESSMENT.....	6
CONCLUSIONS AND RECOMMENDATIONS.....	18
DOCUMENTATION PROVIDED TO EFSA.....	19
REFERENCES.....	20
SCIENTIFIC PANEL MEMBERS.....	23
ACKNOWLEDGEMENT.....	23

BACKGROUND

On 2 July 2004 EFSA received from the German Competent Authority an application (reference EFSA-GMO-DE-2004-03), for authorisation of MON 863 x MON 810 maize (Unique Identifier MON-ØØ863-5 x MON-ØØ81Ø-6), submitted by Monsanto within the framework of Regulation (EC) No 1829/2003 on genetically modified food and feed (EC, 2003).

In the past, EFSA was requested to deliver its opinion on two applications for authorisation of MON 863 x MON 810. The first application, covering food uses, was submitted under the Novel Food Regulation (EC) No 258/97 (EC, 1997). The other application covering feed uses, import and processing was submitted under Directive 2001/18/EC on the deliberate release of genetically modified organisms (GMOs) into the environment (EC, 2001). Both applications concerned placing on the market of MON 863 maize and MON 863 x MON 810 maize. On 2 April 2004 the EFSA GMO Panel issued two opinions concluding on the safety of MON 863 maize (EFSA, 2004a,b). However, the Panel was divided over the need for confirmatory data for the risk assessment of MON 863 x MON 810 maize, in particular the need for an additional 90-day rat study with MON 863 x MON 810 maize. Therefore no agreement could be reached on the safety evaluation of this maize. To resolve this issue, EFSA decided to request the study in question, from Monsanto, in order to allow the Panel to finalise its evaluation².

The applicant provided the 90 days rat study of MON 863 x MON 810 maize on 5 April 2005. After receipt of the full data package, EFSA referred the issue back to the GMO Panel and asked the Panel to conclude the risk assessment of MON 863 x MON 810 maize.

Due to changes in the EU legislation, the applicant had to introduce an application under Regulation (EC) No 1829/2003 (EC, 2003) to replace the application under Regulation (EC) No 258/97 and decided to include in the scope both food and feed uses (reference EFSA-GMO-DE-2004-03). Import and processing are still covered by the scope of the notification under Directive 2001/18/EC (reference C/DE/02/9).

After receiving the application EFSA-GMO-DE-2004-03 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 website³. 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 26 November 2004 EFSA

² http://www.efsa.eu.int/press_room/press_release/385/press_release_0504_gmo_en1.pdf

³ http://www.efsa.eu.int/science/gmo/gm_ff_applications/catindex_en.html

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 26 February 2005) within which to make their opinion known.

In delivering the present opinion the Panel considered the different applications regarding MON 863 x MON 810 maize, the information of the single insert lines MON 863 and MON 810, the additional information provided by the applicant and the specific questions and concerns, raised by the Member States. Although an overall single risk assessment has been made, for regulatory reasons, opinions for the application under Regulation (EC) No 1829/2003 and the notification under Directive 2001/18/EC (EFSA, 2005b) are issued separately.

The single events MON 863 and MON 810 have been the subject of earlier assessments. MON 863 has received EFSA opinions in favour of its authorisation (EFSA, 2004a,b). MON 810 was approved under Directive 90/220/EEC by Commission Decision 98/294/EC (EC, 1998a). The use of food and food ingredients from MON 810 maize was notified in 1997 under Article 5 of Regulation (EC) No 258/97⁴ (EC, 1998b).

The GMO Panel carried out a scientific assessment of the genetically modified maize MON 863 x MON 810 for food and feed use, 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.

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, 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.

⁴ 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' (EC, 1998b) was considered by the UK Advisory Committee on Novel Foods and Processes (ACNFP, 1996).

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 for food and feed uses.

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

The 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

GM maize MON 863 x MON 810 was 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, 2004d). MON 863 x MON 810 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 MON 810. 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 GM hybrids are referred to as MON 863 x MON 810 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 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 characterization

2.1. Issues raised by the Member States

(1) Comments were made regarding the structure and stability of the inserts in MON 863 x MON 810 maize; (2) comments were made regarding the differences in expression of the Cry proteins in MON 863 x MON 810 maize and parental transgenic lines and the variability between different locations.

2.2. Evaluation of relevant scientific data

The EFSA GMO Panel guidance document (EFSA, 2004d) 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 × MON 810 maize and no new genetic modification was involved. The two inserts that are present in MON 863 × MON 810 were derived from maize lines containing two independent events: MON 863 and MON 810. Each of these GM maize events was the subject of an earlier safety evaluation and separate Opinions (EFSA, 2004a,b; SCP 1998b) for each of them have been published. MON 863 × MON 810 combines the insect protection traits from MON 863 and MON 810. Each of the inserts is located on a separate chromosome in the maize genome.

2.2.2. Evaluation of the single events

MON 863

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, 2004c 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).

MON 810

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) 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).

2.2.3. Transgenic constructs in the hybrid

A cross between the two transgenic lines was used to construct the maize MON 863 x MON 810. The molecular structures of the DNA inserts present in MON 863 x MON 810 maize were investigated using Southern analyses. This involved the use of DNA probes for the individual *cry* genes present in MON 810 and MON 863 and genomic DNA digested with *NcoI/EcoRI*, *EcoRV* or *HindIII* for these respective insertion events. The fingerprints detected were consistent with the combination of the MON 810 and MON 863 inserts in MON 863 x MON 810 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 inserts

Expression levels of Cry3Bb1, Cry1Ab, and *NptII* proteins were measured in samples of various maize tissues including kernels from maize hybrids cultivated during field trials in one season (Argentina, 1999-2000). Cultivated maize lines included MON 863 x MON 810, MON 863, and MON 810. Cry3Bb1 and Cry1Ab levels in kernels of MON 863 x MON 810 were on average higher than their levels in the comparator lines MON 863 and MON 810. However, the ranges were broad and there were overlaps between the levels of Cry proteins expressed in the MON 863 and MON 810 parents and in MON 863 x MON 810 maize. This reflects variability in gene expression, which may have been influenced, for example, by environmental factors. In addition it is conceivable that different genetic backgrounds and heterosis had an influence on expression levels. The Panel concludes that these data do not raise safety concerns. In most samples, the *NptII* transgenic protein was undetectable in kernels of MON 863 x MON 810 and MON 863 maize.

2.2.5. Inheritance and stability of inserted DNA

The genetic stability of the inserted DNA in event MON 863 was demonstrated. The genetic stability of MON 810 was established in its original safety assessment (SCP, 1998b) under Council Directive 90/220/EEC (EC, 1990). In MON 863 x MON 810 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 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 MON 863 x MON 810 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.

Cry3Bb1 and Cry1Ab levels in kernels of MON 863 x MON 810 were on average higher than their levels in MON 863 and MON 810. However, the ranges were broad and there were overlaps between the levels of Cry proteins expressed in the MON 863 and MON 810 parents and in MON 863 x MON 810 maize. This reflects variability in gene expression, which may be due to environmental factors or heterosis in the hybrid genetic background. The Panel concludes that these data do not raise safety concerns. In most samples, the NptII transgenic protein was undetectable in kernels of MON 863 x MON 810 and MON 863 maize.

3. Comparative analysis

3.1. Issues raised by the Member States

(1) The hypothetical issue of potential synergistic or unintended effects that might result from hybridization of GM maize varieties was raised; (2) additional comparative analysis of the GM maize and its comparators was recommended.

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 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 between MON 863 and its comparator, which were within the natural range of variation for maize. The Panel thus concluded that these differences were of no biological significance.

In its opinion on maize MON 810 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 non-transgenic control grown during two seasons, this committee concluded that no significant nutritional changes could be detected in maize MON 810. The Panel concurs with the 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 maize was compared with control lines that had not been genetically modified and with commercial hybrids. F₁ generations for MON 863 x MON 810 maize were used for the studies and their self-pollination produced the respective F₂ seed generations, which were the grain material used for the compositional analysis.

Compositional analysis was carried out on forage and grain obtained from field trials in Argentina at four locations with replicates in each location during a single season (1999-2000). The Panel considers that these geographical locations are representative of major countries exporting maize to the EU. 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 MON 810 maize.

3.2.3. Compositional analysis

The concentrations of macro-nutrients, micro-nutrients, and anti-nutrients, as well as secondary metabolites were included in these data. Cultivated maize included MON 863 x MON 810, parental single event GM lines MON 863 and MON 810, a non-transgenic control and commercial lines. In the comparisons of data from all four combined sites, a number of statistically significant differences were observed. The average level of linolenic acid (C18:3) in kernels of MON 863 x MON 810 maize was significantly decreased compared with that of all comparators in each separate location: 1.01 ± 0.02 % of total fatty acids in MON 863 x MON 810 compared with 1.17 ± 0.02 % in MON 863, 1.10 ± 0.02 % in MON 810, 1.19 ± 0.02 % in the control, and 1.19 ± 0.01 % in commercial reference lines. The Panel considers this difference as being small and within the range found in the literature and within the natural variation for linolenic acid (0.8 - 2 % and 0.8 - 1.1 %, respectively). In addition, there are reports that the fatty acid composition of maize kernels can vary substantially between maize varieties, and is influenced particularly by genetic factors (e.g. Dunlap *et al.*, 1995). The Panel concludes, therefore, that the difference in linolenic acid is not meaningful from a biological point of view. In addition, the Panel considers it unlikely that this difference would lead to adverse health effects.

3.2.4. Agronomic traits and GM phenotype

Regarding MON 863 x MON 810 maize, the Panel does not anticipate interactions (*i.e.* synergistic or antagonistic) as a result of the genetic modification which could alter the agronomic characteristics. Furthermore, field trials performed with MON 863 x MON 810 maize did not show any agronomic differences. The Panel accepts the absence of further agronomic data.

3.3. Conclusion

Comparison of MON 863 x MON 810 maize with controls, both single-trait parental lines and various commercial reference hybrids showed statistically significant differences in several compounds. The Panel considers that the levels of these compounds are within normal ranges of variation and there is no need for further assessment in MON 863 x MON 810 maize. Furthermore, based upon these results in addition to those obtained for single-trait parental GM lines MON 863 and MON 810 during multiple seasons, the Panel considers the likelihood of occurrence of unintended effects as negligible.

4. Food/feed safety assessment

4.1. Issues raised by the Member States

(1) Additional toxicity studies were recommended, such as 90-days rat feeding studies, to test for potential synergistic or unintended effects that might result from hybridization of GM maize varieties; (2) extended testing for allergenicity of MON 863 x MON 810 maize was suggested,

including the immunogenicity of transgenic proteins or the unintended alteration of intrinsic allergenicity of maize.

4.2. Evaluation of relevant scientific data

4.2.1. Evaluation of the single events

MON 863

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 proteins 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 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).

MON 810

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

Application EFSA-GMO-DE-2004-03 covers food and feed consisting or derived from the genetically modified maize MON 863 x MON 810. Maize kernels are used mainly for animal feed and to a smaller scale for direct human consumption i.e. sweet maize kernels. Products from maize kernels such as flour, starch and its by-product gluten, syrups, bran and maize germ oil can be regarded as important base materials for food production.

As the modification in MON 863 x MON 810 maize is only intended to improve the agronomic performance but not to influence nutritional aspects, production processes and overall use of maize as a crop are not expected to be influenced as a result of the introduction of the GM plants to the market.

4.2.3. Stability during processing

Based on the data of the compositional analysis of the raw agricultural commodities of MON 863 x MON 810 maize and the non-GM maize, the Panel is of the opinion that there are no reasons to

assume that the stability of the processed products derived from this maize would be different from the non-GM processed products.

4.2.4. Toxicology

4.2.4.1. Toxicological assessment of expressed novel proteins in MON 863 x MON 810 maize

In previous evaluations (SCP,1998b; EFSA, 2004a,b) the safety of Cry3Bb1 and Cry1Ab proteins has been shown by testing their *E. coli* equivalents for rapid digestion *in vitro* simulated gastric fluids and for lack of treatment-related toxicity of both proteins in a mouse acute gavage study at doses levels higher than those encountered in human or animal diets.

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 and therefore no further safety assessment of new constituents in MON 863 x MON 810 maize is warranted.

4.2.4.3. Toxicological assessment of the whole GM food/feed

Subchronic oral toxicity

Maize lines MON 863 and MON 810 were tested separately for toxicity as part of the diet for rats in 90-day studies. The results of these 90-day rodent studies do not indicate adverse effects resulting from the consumption of maize lines MON 863 and MON 810.

The Panel accepts that there are valid scientific arguments for the use of data provided for the single insert lines for the safety assessment of MON 863 x MON 810 maize. Given the specific modes of action of the inserted Cry3Bb1 and Cry1Ab proteins, there is no expectation that the Cry proteins expressed in these plants would have pleiotropic effects either in isolation or in combination. However, an additional 90-day rat study with MON 863 x MON 810 maize was requested by EFSA in order for the Panel to finalise its opinion.

The safety of the whole product derived from kernels of MON 863 x MON 810 maize was tested for in a 90-days toxicity study with rats. The design and execution of this study complied with OECD Guideline 408 (OECD, 1998). Three groups of rats consisting of 20 rats per sex within each group received maize-containing diets *ad libitum* for 90 days. One group received a diet containing 33% MON 863 x MON 810 maize. Another group received a diet containing 11% MON 863 x MON 810 maize, supplemented with 22% control maize. A concurrent control group was administered a diet containing 33% control maize.

All animals were examined daily for appearance, morbidity, and mortality. In addition, they were examined weekly for additional physical characteristics. 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.

Small deviations in food consumption by females on test diets containing MON 863 x MON 810 were observed as compared with those on the control diet. However, these observations did not

occur consistently throughout the experimental period. Neither did they occur in a dose-related manner, nor were they observed in both sexes. Therefore, the Panel considers the observed differences neither as related to the test diet nor as being relevant toxicologically. No effects were observed on body weight gain.

Most of the clinical chemistry data showed no differences or were below the limit of detection. Nevertheless, analysis of the clinical chemistry and pathology data showed statistically significant decreases in mean corpuscular haemoglobin concentration in male animals in the 11% and 33% test diet groups. Values of 32,7 g/dl for the control group, 31,6 g/dl for the 11% group and 31,6 g/dl for the 33% group were measured and therefore these values were not dose-related. Furthermore, there were no changes in the red blood cell counts, haematocrit values, and other red blood cell parameters. Another statistically significant, but slight difference in basophil counts was observed but only in males that received the 11% test diet. The Panel considers the changes observed to be of no toxicological relevance.

Concerning organ weights, some statistically significant differences were observed. For example, lower mean absolute and relative thyroid/parathyroid weights compared with the control group (0,257 g) were observed in female animals of groups fed the 11% (0,200 g) and 33% (0,219 g) test diets. However, this difference did not exhibit a dose-response relationship and microscopic observations showed no abnormalities either. The mean kidney weight relative to body weight was statistically significantly lower in females in the 33% test diet group. The Panel considers this as an accidental finding, since the mean absolute kidney weight and kidney relative to brain weight was not affected. Apart from these differences, microscopic examination of thyroid/parathyroid and kidneys, as well as urine analysis showed no effects related to feeding rats with the test diets containing MON 863 x MON 810 maize as compared with 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, 2004d; CAC, 2003).

4.2.5.1. Assessment of allergenicity of newly expressed proteins Cry1Ab and Cry3Bb1

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, 2004d; 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, 2005a) and approved (EC, 1998a,b,c; EC, 1999; EC, 2004). 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 allergenic 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.*, 1999a; 2000). This demonstrates that Cry1Ac has no or low allergenic potential. This is also supported by recent bioinformatic studies carried out by the Swedish National Food Administration using a newly developed methodology (Soeria-Atmadja *et al.*, 2004; Bjorklund *et al.*, 2005) showing the absence of sequence homology between Cry1Ac and known allergens (unpublished results).

In the same manner, Cry1Ab has been shown to act as an adjuvant e.g. it enhances the mucosal and/or the systemic antibody response to a protein which is co-administered with the Cry protein (Vazquez *et al.*, 1999b; Moreno-Fierros *et al.*, 2003). 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

Risk assessment of the whole GM plant must consider whether allergenicity of the whole crop could be increased as an unintended effect of the random insertion of the transgene in the genome of the host, for example through qualitative or quantitative modification of the pattern of expression of endogenous proteins. Such unintended effects may occur at each genetic modification (*i.e.* in MON 810 and in MON 863) but also in the double transgenic plant after crossbreeding of MON 810 and MON 863. This issue does not appear relevant to the Panel, however, 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

MON 863, MON 810, and MON 863 x MON 810 maize have been studied in separate nutritional feeding studies with broilers. These animals grow rapidly to full size within six weeks and are therefore a sensitive model with which to detect any nutritional imbalances that might be present in the GM maize lines. Both performance (weight gain, feed consumption) and carcass parameters (weight, weight of carcass parts and compositional analysis of breast and thigh meat) were measured. None of these studies showed adverse effects in animals fed the test diets.

The Panel considers that these data are sufficient to conclude that there is no reason to assume that nutritional properties of maize MON 863, MON 810 and MON 863 x MON 810 would be different from those of conventional maize.

4.2.7. Post market monitoring of GM food/feed

MON 863 x MON 810 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 Cry3Bb1, Cry1Ab and NptII proteins.

The results of 90-day sub-chronic rodent studies do not indicate adverse effects from consumption of maize lines MON 863 x MON 810 and the Panel concludes that there are no resultant concerns over its safety. The Panel considers that the data from the 90-day rat feeding study with grain from MON 863 x MON 810 maize are sufficient to conclude that there is no reason to assume that the MON 863 x MON 810 is different from the 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 and Cry3Bb1 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, MON 810, and MON 863 x MON 810 maize have been studied in separate 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, MON 810 and MON 863 x MON 810 would be no different from those of conventional maize.

5. Environmental risk assessment and monitoring plan

5.1. Issues raised by the 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 via organic waste material and litter or sewage, which occur during processing or through spillage; 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. MON 810 was assessed for import and cultivation (SCP, 1998b; EFSA, 2004a,b).

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.

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 maize has no altered survival, multiplication or dissemination characteristics except in the presence of Cry proteins. 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 MON 810 maize and traditionally bred maize.

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, 2004c), 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 *cry1Ab* 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 cry 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 from MON 863 x MON 810 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 MON 863 x MON 810 maize. The EFSA GMO Panel recently formulated an Opinion (EFSA, 2004c) 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, 1998a, b).

b) Plant to plant gene transfer

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.

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.

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 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 MON 810 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 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 and Regulation (EC) No 1829/2003.

CONCLUSIONS AND RECOMMENDATIONS

The GMO Panel assessed MON 863 x MON 810 maize which is produced by a cross between inbred lines of maize containing MON 863 and MON 810 events. The MON 863 and MON 810 maize were evaluated previously (EFSA, 2004a,b and SCP, 1998b) and MON 810 has been authorized (EC, 1998a,b). In assessing MON 863 x MON 810 maize, both the single insert lines and MON 863 x MON 810 maize were considered. The Panel concluded that it was acceptable to use data for the single insert lines MON 863 and MON 810 in support of the safety assessment of MON 863 x MON 810 maize.

The results of 90-day sub-chronic rodent study do not indicate adverse effects from consumption of maize MON 863 x MON 810 maize and the Panel concludes that there are no resultant concerns over its safety. The Panel considers that the data from the 90-day rat feeding study with grain from MON 863 x MON 810 maize are sufficient to conclude that there is no reason to assume that MON 863 x MON 810 maize is different from the conventional maize regarding its safety.

In conclusion, the Panel considers that the information available for MON 863 x MON 810 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 German Competent Authority (Bundesamt für Verbraucherschutz und Lebensmittelsicherheit), dated 1 July 2004 concerning the submission to EFSA of application MON 863 x MON 810 maize within the framework of Regulation (EC) No 1829/2003.
2. Letter from EFSA to applicant, dated 27 August 2004, concerning a request for further information for application EFSA-GMO-DE-2004-03 on MON 863 x MON 810 maize submitted under Regulation (EC) No 1829/2003 (Ref. SR/sp (2004) 608).
3. Letter from applicant to EFSA, dated 2 November 2004, providing further information for application EFSA-GMO-DE-2004-03 on MON 863 x MON 810 maize submitted under Regulation (EC) No 1829/2003.
4. Letter from EFSA to applicant, dated 26 November 2004, concerning the “Statement of Validity” for application EFSA-GMO-DE-2004-03 on MON 863 x MON 810 maize submitted under Regulation (EC) No 1829/2003 (Ref. SR/KL/jq (2004) 1025) and requesting additional information.
5. Submission of the application EFSA-GMO-DE-2004-03 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
6. The following application dossiers concerning MON 863 x MON 810 maize including assessment reports, the respective Member States comments/objections and additional information were considered where appropriate:
 - a. Notification (C/DE/02/9) to market products containing genetically modified organisms in accordance with Directive 2001/18/EC submitted by Monsanto to EFSA on 2 November 2004.
 - b. Application for placing on the market of novel foods and novel food ingredients containing genetically modified organisms in accordance with Regulation (EC) No 258/97 submitted by Monsanto to EFSA on 2 November 2004.
7. Letter from EFSA to applicant, dated 9 February 2005, to stop the clock on behalf of JRC-CRL for application EFSA-GMO-DE-2004-03 on MON 863 x MON 810 maize submitted under Regulation (EC) No 1829/2003 (Ref. SR/MR/jq/ (2005) 160).
8. Letter from applicant to EFSA, dated 5 April 2005, providing a 90 day feeding study in rats for application EFSA-GMO-DE-2004-03 on MON 863 x MON 810 maize submitted under Regulation (EC) No 1829/2003.
9. Letter from EFSA to applicant, dated 13 April 2005, concerning a request for a monitoring plan for application EFSA-GMO-DE-2004-03 on MON 863 x MON 810 maize submitted under Regulation (EC) No 1829/2003 (Ref. SR/AC/jq (2005) 428).

10. Letter from applicant to EFSA, dated 14 April 2005, providing the monitoring plan for application EFSA-GMO-DE-2004-03 on MON 863 x MON 810 maize submitted under Regulation (EC) No 1829/2003.
11. 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. Report on processed products from GM insect-resistant maize. Annual Report 1996, Appendix IV. Advisory Committee on Novel Foods and Processes, UK.
http://www.foodstandards.gov.uk/multimedia/pdfs/acnfp_app_i-vi.pdf
- Ahmad, A., Wilde G.,E., Zhu K., Y., 2005. Detectability of Coleopteran-specific Cry 3Bb1 protein in soil and its effect on nontarget surface and below-ground arthropods. *Environmental Entomology*, 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. *Official Journal of the European Communities*, L117, 15-27.
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. *Official Journal of the European Communities* L43, 1-7.
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. *Official Journal of the European Communities* L131, 32-33.

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

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

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

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. Official Journal of the European Communities, L106, 1-39.

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

EC, 2004. 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). Official Journal of the European Union L300, 48-51.

http://europa.eu.int/eur-lex/lex/LexUriServ/site/en/oj/2004/l_300/l_30020040925en00480051.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. EFSA Journal 49, 1-25

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. EFSA Journal 50, 1-25.

http://www.efsa.eu.int/science/gmo/gmo_opinions/383/opinion_gmo_07_en1.pdf

EFSA, 2004c. 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/opinion_gmo_05_en1.pdf

- EFSA, 2004d. 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 (2004) 99, 1-94.
http://www.efsa.eu.int/science/gmo/gmo_guidance/660_en.html
- EFSA, 2005a. 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. EFSA Journal (2005) 213, 1-33.
http://www.efsa.eu.int/science/gmo/gmo_opinions/922/gmo_opinion_ej213_bt11maize_cultivation_en1.pdf
- EFSA, 2005b. 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 x MON 810, for import and processing, under Part C of Directive 2001/18/EC from Monsanto, The EFSA Journal (2005) 251, 1-22.
http://www.efsa.eu.int/science/gmo/gmo_opinions/1030_en.html
- Metcalfe, D.D., Astwood, J.D., Townsend, R., Sampson, H.A., Taylor, S.L. and Fuchs, R.L., 1996. Assessment of the allergenic potential of foods derived from genetically engineered crop plants. Crit. Rev. Food. Sci. Nutr., 36(S), 165-186.
- Moreno-Fierros, 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. and Strikema, W.J., 1992. Biosafety of kanamycin-resistant transgenic plants: an overview. Transgenic Crops, 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
- 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
- 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
- SCP, 1998c. Opinion of the Scientific Committee on Plants on the Genetically Modified Maize Lines Notified by the Novartis Company (Notification C/GB/96/M4/1).
http://europe.eu.int/comm/food/fs/sc/scp/out05_en.html

- SCP, 2000. Opinion of the Scientific Committee on Plants on the submission for placing on the market of genetically modified insect resistant and glufosinate ammonium tolerant (Bt-11) maize for cultivation. Notified by Novartis Seeds SA Company (notification C/F/96/05-10). http://europa.eu.int/comm/food/fs/sc/scp/out86_gmo_en.html
- Soeria-Atmadja D., Zorzet A., Gustafsson M.G., Hammerling U., 2004. Statistical Evaluation of Local Alignment Features Predicting Allergenicity Using Supervised Classification Algorithms. *Int. Arch. Allergy Immunol.*, 133(2),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.