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[1] (Question No EFSA-Q-2003-121)

Opinion adopted on 2 April 2004

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 and the maize hybrid MON 863 x MON 810. The opinion is based on two questions raised by the Commission related to applications for the placing of the maize on the market by Monsanto under the Novel Food Regulation (EC) No 258/97 (EC, 1997) and the Directive 2001/18/EC on the deliberate release of genetically modified organisms (GMOs) into the environment (EC, 2001).

In the first question, the GMO Panel was asked to consider the safety of foods and food ingredients derived from MON 863 and MON 863 x MON 810 maize. In the second question the GMO Panel was requested to consider whether there is any scientific reason to believe that the placing on the market of MON 863 and MON 863 x MON 810 maize, for import and processing, is likely to cause any adverse effects on human health and the environment. The questions followed two separate scientific assessments which were initially made by the competent authorities of Germany and subsequently evaluated by all other Member States. An assessment of the MON 863 and MON 863 x MON 810 maize was requested by the Commission because of questions raised by several Member States following the evaluations at the national level. When this is the case, EU legislation requires that EFSA carries out a further assessment and provides an opinion.

In delivering its opinion the Panel considered the applications and additional information provided by the applicant and the specific questions and concerns raised by the Member States. At the request of the Commission, the Panel has provided two separate opinions. However, as both dossiers cover to a large extent the same issues a single risk assessment is provided for both opinions.

[1] For citation purposes: 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, The EFSA Journal (2004) 50, 1-25

The maize MON 863 and the hybrid MON 863 x MON 810 were assessed with reference to their intended use and the appropriate principles described in the guidance document for the risk assessment of genetically modified plants and derived food and feed (EC, 2003). The scientific assessment included the transformation process, the vectors used and the transgenic constructs in the genetically modified plant. Furthermore 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 toxicology 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. The hybrid maize MON 863 x MON 810 was produced by a conventional cross between inbred maize lines MON 863 and MON 810 to combine the rootworm resistance trait in MON 863 with the trait present in MON 810 protecting against lepidopteran pests (*Ostrinia nubilalis* and *Sesamia* spp.). MON 810 maize was approved under Directive 90/220/EEC (EC, 1990) by Commission Decision 98/294/EC (EC, 1998a). The use of food and food ingredients from MON 810 maize was notified in 1997 under the Regulation (EC) 258/97[2].

Molecular analysis of MON 863 maize demonstrated that only the two expected full-length proteins, Cry3Bb1 and NptII, would be encoded by the insert. With respect to the presence of an intact *npt*II gene, the GMO Panel recently formulated an opinion (EFSA, 2004) on antibiotic resistance genes in GM plants and concluded that the use of *npt*II as a selection marker did not pose a risk to the environment nor to human and animal health. DNA sequences at the junctions between the insert and parent genome were determined revealing the presence of mitochondrial DNA at both flanks. The molecular analysis does not differentiate between the integration of insert DNA within a region of mitochondrial DNA that is already present in the nuclear genome and the acquisition of this organelle DNA as part of the primary integration during transformation. The integration of organellar DNA within plant nuclear genome is established as a normal phenomenon and the Panel considers that the resolution of this distinction would not significantly impact on the present safety assessment. Analysis of DNA sequences spanning the junctions identified open reading frames. 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. For MON 810 maize, junctions between the insert and the plant genome were delineated and the complete DNA sequence of the insert determined. An apparent inconsistency between the original dossier and newly provided information was resolved. Investigation of the molecular structures of the DNA inserts in MON 863 x MON 810 hybrid confirmed that insert structures and loci of insertion were retained.

Compositional analyses of kernels from MON 863, a non-modified control and commercial lines revealed minor differences in some plant constituents, which were not considered to be biologically significant. Comparison of MON 863, MON 810 and MON 863 x MON 810 hybrid showed a statistically significant difference in the copper content, which is not unexpected given the distinct genetic backgrounds of the single-

[2] 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).

insert plants and the hybrid. Since the copper levels are within normal ranges of variation, the Panel considers that there is no need for further assessment in the hybrid.

Cry3Bb1 and Cry1Ab levels in kernels of MON 863 x MON 810 hybrid were higher than in MON 863 and MON 810. The ranges were broad and showed overlap between the double- and single-trait hybrids. The Panel concludes that these data do not raise safety concerns.

The Cry3Bb1 protein produced in *E. coli* was considered by the Panel as equivalent to the plant-derived protein and an acceptable alternative for use in the toxicological testing. Adequate acute toxicity data were provided for both Cry3Bb1 and NptII proteins. An allergy risk evaluation of the Cry1Ab and Cry3Bb1 proteins was carried out from which it was concluded that the probability of allergenicity was very low.

The results of 90-day sub-chronic rodent studies do not indicate adverse effects from consumption of MON 863 and MON 810 and the Panel concludes that there are no concerns over their safety.

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

The notification C/DE/02/9 only concerns 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 Panel concludes that the amounts of Cry toxin being distributed onto land in manure 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 addresses the outstanding questions raised by the Member States and considers that MON 863 will not have an adverse effect on human and animal health or the environment in the context of its proposed use. In the case of the hybrid MON 863 x MON 810, while it was considered that it is scientifically valid to use data from the single GM lines MON 863 and MON 810 to support the safety assessment of the hybrid MON 863 x MON 810, the Panel was divided over the need for confirmatory data for the safety assessment of the hybrid, in particular, the need for an additional 90-day rat study with MON 863 x MON 810. Therefore the Panel could not reach agreement on the safety evaluation of the hybrid.

Key words: GMOs, maize, MON 810, MON 863, MON 863 x MON 810, insect protection, Cry3Bb1, Cry1Ab, NptII, food safety, feed safety, human health, environment, import, Regulation (EC) 258/97, Directive 90/220/EEC, Directive 2001/18/EC.

TABLE OF CONTENTS

BACKGROUND

On 13 August 2002, Monsanto submitted a request under Article 4 of the Novel Food Regulation (EC) N° 258/97 (EC, 1997) to the German competent authorities for placing on the market foods and food ingredients derived from MON 863 and MON 863 x MON 810 insect-protected maize.

On 8 April 2003, the German competent authorities forwarded to the Commission its initial assessment report, carried out by the Robert-Koch-Institut (DE), which had reached the conclusion that an additional assessment was required because of the presence of an antibiotic resistance marker gene (*npt*II) used in the product concerned.

On 3 June 2003, the Commission forwarded the initial assessment report to the other Member States. Several Member States supported the conclusion of the initial assessment report carried out by the German competent authorities. Other Member States submitted additional comments/objections.

In consequence, a Community Decision is now required under Article 7, paragraph 1 of Regulation (EC) No 258/97. Beforehand, and in view of the questions raised in the initial assessment report by the German authorities and by other Member States and the Community interest in this matter, the European Commission has decided to seek the opinion of the European Food Safety Authority (EFSA) as there might be an effect on public health (Article 11 of Regulation (EC) No 258/97).

EFSA was asked to provide a scientific opinion within 3 months. The evaluation by EFSA started on 10 December 2003 after receipt of the full background information (request from the Commission, dossier of the applicant and objections of Member States). During the evaluation period, EFSA requested further clarifications from the applicant; this procedure, in agreement with the Commission, extended the final deadline set for the delivery of this opinion.

TERMS OF REFERENCE

In accordance with Article 29 (1) (a) of Regulation (EC) No 178/2002 (EC, 2002), the European Food Safety Authority is asked to carry out the additional assessment for MON 863 and MON 863 x MON 810 insect-protected maize in the context of Regulation (EC) N° 258/97 concerning the use of grains and grain derived food ingredients as required in the initial assessment report by the German Competent Authority[3].

EFSA is asked to consider the elements of a scientific nature in the comments/objections raised by the other Member States.

EFSA is not requested to give an opinion on the non-scientific objections raised by competent authorities in their replies, in the context of the entry into force of forthcoming legislation or requests for further legislative/implementing measures.

ASSESSMENT

1. Introduction

GM maize MON 863 and the hybrid GM maize MON 863 x MON 810 were assessed with reference to their intended use and the appropriate principles described in the guidance document for the risk assessment of genetically modified plants and derived food and feed (EC, 2003). The hybrid MON 863 x MON 810 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 the GM hybrid line is referred to as MON 863 x MON 810. The combination of separate inserts as a result of a conventional cross between GM plants raises questions about the extent to which data on the individual GM plant lines can be extrapolated to assess the hybrid. The Panel regards this as a case-by-case issue in which the detail of the individual inserts is of particular relevance. In addressing the MON 863 x MON 810 hybrid the Panel does not set a precedent for the future safety assessment of other GM hybrid lines.

2. Molecular characterization

2.1. Issues raised by the Member States

Directive 2001/18/EC

Within the framework of the Directive, some Member States indicated the following objections after the applicant had answered their first questions. The retention of the antibiotic resistance gene *nptII* coding for kanamycin and neomycin resistance was not accepted. For MON 863, the DNA sequence data at the flanks of the insert were considered insufficient on grounds that unintended changes were incompletely identified and characterized. In particular, there was concern that this data did not

[3] Additional assessment in accordance with Article 6(3) of Regulation (EC) N° 258/97

extend to regions of plant genome DNA on both flanks. There was concern that the presence of organelle DNA insertion events throughout the genomes of MON 863 and MON 810 had not been investigated. In the latter case the effectiveness of backcrossing to reduce the presence of any such sequences was questioned. There were apparent inconsistencies between the early and the most recent data on the bioinformatic analysis of DNA sequences at the 5' flank of the insert in MON 810.

Regulation (EC) 258/97

In addition to the above objections, Member States expressed the following concerns in the framework of the safety assessment under the Regulation (EC) 258/97. The limited data for the hybrid MON 863 x MON 810 was considered inadequate and in particular a complete analysis including the insert flanking sequences was requested.

2.2. Relevant background data

2.2.1. The transformation process and vector constructs

MON 863

The genetically modified maize MON 863 was generated by transformation of *Zea mays* cell culture line AT824 (initiated from immature embryos of an inbred maize line AT) with a *Mlu*I restriction fragment from plasmid PV-ZMIR13 using particle acceleration technology. The DNA fragment used for transformation carried two expression cassettes; a selectable marker gene *nptII* encoding neomycin phosphotransferase II and a trait gene encoding a variant *Bacillus thuringiensis* Cry3Bb1 insecticidal protein (Crickmore *et al*., 1998). The *nptII* gene was regulated by the 35S promoter and used the NOS 3' polyadenylation sequence as terminator. Regulation of the variant *cry3Bb1* gene involved: a CaMV 35S promoter containing four tandem copies of the AS-1 element (Lam and Chua, 1990); the untranslated 5'mRNA leader sequence of the wheat major chlorophyll a/b-binding protein; the rice actin intron (ract1); a 3' nontranslated region of the wheat heat shock protein 17.3 which terminates transcription and directs polyadenylation. The linear DNA fragment used for transformation was prepared by digestion of plasmid PV-ZMIR13 with restriction endonuclease *Mlu*I, separation of the fragments by agarose gel electrophoresis and isolation of the DNA fragment that encoded the *cry3Bb1* and *nptII* expression cassettes. Thus, the *Mlu*I fragment used for transformation was not expected to contain plasmid backbone DNA sequences.

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 insecticidal activity against corn rootworm. The maize hybrid MON 863 x MON 810 was produced by conventional breeding to combine the rootworm resistance trait in MON 863 with the lepidopteran insect resistance trait present in another GM maize, MON 810. The latter was approved under Directive 90/220/EEC by Commission Decision 98/294/EC (EC, 1998) and it has been grown commercially since 1997 in the USA, Canada, Argentina and South Africa. The use of food and food ingredients from MON 810 maize was notified in 1997 under the Regulation (EC) 258/97.

Genetically modified maize MON 810 was generated by transformation of *Zea mays* with plasmids PV-ZMBK07 and PV-ZMGT10 using particle acceleration technology. Subsequent molecular characterization demonstrated that sequences derived from plasmid PV-ZMBK07 were inserted in MON 810. These included a partial enhanced CaMV 35S (e35S) promoter, the maize HSP70 intron (ZmHSP70), and sufficient of the

cry1Ab coding region to encode an insecticidally active Cry1Ab protein. There was no evidence that any other portion of plasmid PV-ZMBK07 DNA was integrated into the maize genome and there was no evidence that any portion of plasmid PV-ZMGT10 is present in MON 810.

Presence of the 35S CaMV promoter

The 35S CaMV promoter was evaluated during the safety assessment of genetically modified maize NK603 (EFSA, 2003) and the Opinion concluded that the use of this promoter in GM plants was acceptable.

Presence of the marker gene nptII encoding neomycin phosphotransferase II

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 transformed GM plants. The EFSA GMO Panel recently formulated an Opinion (EFSA, 2004) 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).

2.2.2. Transgenic constructs in the genetically modified plant

Maize event MON 863

The GM plant MON 863 was subjected to molecular analysis in order to determine the insert number (number of integration sites within the maize genome), the copy number (the number of copies of the DNA fragment used for transformation that were inserted in the GM plant), the integrity of the inserted cassettes and the absence of backbone sequences.

Southern blot analyses were undertaken using a variety of DNA probes that included the whole plasmid PV-ZMIR13, the linear *Mlu*I restriction endonuclease fragment used in transformation, the two intact coding regions, their respective promoters, introns, and polyadenylation sequences, and the plasmid backbone. The data obtained demonstrated that event MON 863 contains a single DNA insertion with one copy of both the *cry3Bb1* and the *nptII* cassettes. No additional elements from the DNA fragment used in transformation were detected in the genome of event MON 863.

PCR analysis and DNA sequencing were used to establish a detailed insert structure and to verify the 5' and 3' junction sequences of the insert with the plant genome. This demonstrated the intactness of the 5' and 3' ends of the inserted cassettes. The data confirmed that the MON 863 event does not contain any detectable backbone sequences from plasmid PV-ZMIR13, including the plasmid origin of replication *ori*-pUC and the second *nptII* coding region regulated by a bacterial promoter. These molecular analyses of the transgenic DNA present in maize event MON 863 suggested that only the two expected full-length proteins, Cry3Bb1 and NptII, would be expressed by the insert.

DNA sequences at the junctions between the insert and parent genome were determined. At the 3' flank, approximately 350bp of DNA adjacent to the insert was sequenced initially and bioinformatic analysis indicated a high degree of homology (>95%) to maize genomic DNA. During the evaluation process, additional sequence data were provided to extend the region of flanking DNA at the 3' end of the insert to 650bp. In addition, a new bioinformatic analysis of the Monsanto database was undertaken leading to the conclusion that this flanking DNA was 100% homologous to the maize mitochondrial genome. At the 5' flank, approximately 500bp of DNA adjacent to the insert was sequenced originally and bioinformatic analysis indicated a high degree of homology (>95%) to mitochondrial DNA, specifically to *Zea mays* NADH dehydrogenase subunit 4 (complex I) gene *nad*4, exon 4. During the evaluation process further data were provided extending the sequenced region at the 5' flank to 1000bp. This additional sequence was 100% homologous to the maize mitochondrial genome. Thus both flanks of the insert DNA were defined as mitochondrial DNA.

Several Member States questioned the adequacy of the original DNA sequence data. The fact that mitochondrial DNA sequences were found in the nuclear genome is not surprising as equivalent observations have been made for conventional plants from a variety of different species, including maize (Adams *et al*., 1999; 2000; Braun *et al*., 1994; Daley *et al*., 2002; Figueroa *et al*., 1999a; 1999b; Fukuchi *et al*., 1991; Goff *et al*., 2002; Kemble *et al*., Kubo *et al*., 2001; 1983; Sun and Callis, 1993). The same is true for plastid DNA. For this reason, the Panel does not support the request from one Member State to investigate the secondary integration of plastid DNA throughout the nuclear genome. However, the molecular analysis at both the 5' flank and the 3' flank of the MON 863 event does not differentiate between the integration of insert DNA within a region of mitochondrial DNA that is already present in the nuclear genome and the acquisition of this organelle DNA as part of the primary integration during transformation.

A bioinformatic analysis of DNA sequences spanning the 5' and 3' junctions of the insert was undertaken (see below) to determine if open reading frames (ORFs) were created by the insertion of DNA into the maize genome. Identified ORFs were analyzed to test for the creation of a potential peptide with homology to known allergens, toxins or proteins that display adverse health effects. These were not found. The specificity of gene expression makes it unlikely that intact mitochondrial ORFs would be functional in the context of the nuclear genome. Organelle DNA insertions appear to occur randomly and there may be cases where the site of insertion interferes with the expression of nuclear genes (Sun and Callis, 1993). For this reason, there is potential for the creation of fusion proteins. However, the frequent acquisition of organelle DNA by the plant nuclear genome in conventional plants means that this is not unique to GM plants (Adams *et al*., 2002; Mackenzie and McIntosch, 1999). In addition, understanding of nuclear and mitochondrial gene expression makes it unlikely that a contiguous tract of mitochondrial DNA would lead to the expression of a protein by a fused ORF. For potential transcripts reading out from the mitochondrial DNA the promoter specificity would prevent the initiation of transcription from a mitochondrial promoter. For transcripts initiated in the nuclear plant genome and extending into the mitochondrial DNA, the probability of gene expression would be low due to the need for a correct mRNA structure. In addition, the molecular data provided eliminate the possibility that additional fragments of insert DNA are present, either associated with the primary insertion event or elsewhere in the genome as a result of secondary insertion events. Moreover, the Panel is reassured by the availability of a 90-day sub chronic toxicity study using MON 863 maize fed to rats (see below), which provides evidence that no harmful novel proteins have been created. Additionally, whole food studies including a 42 day broiler chicken study, and studies on lactating dairy cows (Grant *et al*., 2003) and beef cattle for fattening (Vander Pol *et al*., 2003; Wilson *et al*., 2003) have demonstrated the

wholesomeness of MON 863 maize. In conclusion, the Panel accepts that safety concerns related to the presence of organelle DNA in MON 863 are equivalent to those in conventional plants. Data from animal feeding experiments using MON 863 give added reassurance.

Maize event MON 810

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. Additional experiments were undertaken in order to delineate the junctions between the insert and the surrounding genomic DNA sequences in event MON 810. 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 part of the *cry1Ab* coding region sufficient to encode an insecticidal Cry1Ab protein.

In addition to describing the genomic DNA sequences surrounding the insert, it was determined that there are 2448 base pairs of the 5' portion of the *cry1Ab* coding region in event MON 810.

Some Member States questioned the adequacy of this data and an apparent inconsistency in the 5' flanking gene homology presented in the original dossier for MON 810 and newly provided information. With respect to the latter point, the apparent inconsistency is explained by the fact that databases are constantly updated and thus new matches will be found when homologies are rechecked. For the most recent data, the sequence corresponding to the accession number BZ807454 showed the highest homology with the 244 bp DNA sequence flanking the 5' end of the insert and this database entry was introduced in March 2003. The sequence was not present in the database at the time of earlier homology searches and the key point is that the 244 bp flanking sequence identified by Monsanto matches completely the corresponding sequence identified by Holck *et al*., (2002). Thus, the most recently provided information concerning the 5' end flanking gene homology is consistent with both the original dossier for MON 810 and with the results published by Holck *et al*. (2002).

Data from third party molecular analyses of MON 810 have generated questions about the authenticity or the stability of inserted DNA[4]. It has clearly been established that MON 810 carries a single integrated DNA fragment with a single copy of the 35S promoter, the *hsp*70 intron and the *cry1Ab* gene. Importantly, a less than full length *cry1Ab* gene is incorporated and this conclusion is supported by a Southern analysis demonstrating removal of the *Eco*RI site between the *cry1Ab* open reading frame and the *nos* terminator that was present on the original plasmid used for transformation. This indicates truncation of the *cry1Ab* ORF, a conclusion that is confirmed by DNA sequencing. Whilst this molecular structure for MON 810 is established, a recent poster presentation[5] was brought to the attention of EFSA suggesting that a secondary integration of the *nos* terminator had also occurred in MON 810. This was not reported in the dossier considered by the EFSA GMO Panel and the apparent inconsistency required clarification. During the evaluation process the applicant provided new data in which an appropriate DNA probe was used to specifically investigate the presence of the

[4] See minutes of the 5th Plenary Meeting of GMO Panel: http://www.efsa.eu.int/science/gmo/gmo_meetings/168/minutes_gmo_05_final_en1.pdf

[5] Collonier C, Berthier G, Boyer F, Duplan M-N, Fernandez S, Kebdani N, Kobilinsky A, Romanuk M, Bertheau Y. Characterization of commercial GMO inserts: a source of useful material to study genome fluidity. Poster presented at ICPMB: International Congress for Plant Molecular Biology (n°VII), Barcelona, 23-28th June 2003.

nos terminator in the genome of MON 810. This confirmed the original conclusion that a secondary integration event had not taken place in MON 810.

Maize hybrid MON 863 x MON 810

A conventional cross between the two transgenic lines was used to construct the maize hybrid MON 863 x MON 810. The molecular structures of the DNA inserts present in the hybrid 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 *Nco*I/*Eco*RI or *Eco*RV for these respective insertion events. The fingerprints detected were consistent with the combination of the MON 810 and MON 863 inserts in the hybrid MON 863 x MON 810. Some Member States were not satisfied that this was a sufficiently detailed characterisation and in particular were concerned about possible cross-reaction between the two transgenes derived from MON 863 and MON 810. During the assessment process the applicant provided additional data from new Southern hybridisation experiments in which genomic DNA from maize hybrid MON 863 x MON 810 was digested with restriction endonuclease *Hind*III. This additional analysis confirmed that both gross insert structures and the locus of insertion were retained in the hybrid maize line.

2.2.3. Inheritance and stability of inserted DNA

The genetic stability of the inserted DNA in event MON 863 was demonstrated by Southern blot analysis on genomic DNA from nine generations using the full-length *nptII* coding region as a probe. No differences in banding pattern were observed between the DNA from any of the generations demonstrating the stability of the inserted DNA. Segregation data for the MON 863 Cry3Bb1 trait was studied using Chi square analysis of Mendelian inheritance data over five generations. This demonstrated the heritability and stability of the *cry3Bb1* gene in MON 863. Data support the presence of a single insertion that segregates according to Mendel's laws of genetics and the stability of the insert has been demonstrated through three generations of cross-fertilization and two generations of self-pollination. The genetic stability of MON 810 was established in its original safety assessment under Council Directive 90/220/EEC (SCP, 1998b). The two inserts are combined when hybrid MON 863 x MON 810 seed is produced and this material is not used for seed multiplication. The stability of each insert has been demonstrated in the separate MON 810 and MON 863 inbred lines. The Panel is content that the intended agronomic use of hybrid maize does not lead to the maintenance of the combined inserts in additional generations.

2.2.4. Conclusion

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 *npt*II as a selection marker did not pose a risk to the environment or to human and animal health (EFSA 2004). DNA sequences at the junctions between the insert and parent genomes were determined and bioinformatic analysis revealed the presence of mitochondrial DNA at both the 5' and 3' flanks. The integration of organelle DNA within the nuclear plant genome is established as a normal phenomenon in plant biology and the Panel considered that the resolution of this distinction 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.

For maize line MON 810 additional experiments were undertaken to delineate the junctions between the insert and the plant genome and a complete DNA sequence of the insert was determined, confirming its predicted structure. 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.

The molecular structures of the DNA inserts in the hybrid MON 863 x MON 810 were investigated using Southern analyses and this confirmed that gross insert structures and loci of insertion were retained.

3. Comparative Analysis

3.1. Issues raised by the Member States

Directive 2001/18/EC

The absence of agronomic data for the maize hybrid MON 863 x MON 810 was questioned. The fact that synergistic effects are not anticipated was not accepted as a reason for failing to provide data.

Regulation (EC) 258/97

In addition to the above there was a request for data to be provided for each individual field trial. The concern was raised that, in view of the absence of data for the hybrid MON 863 x MON 810, compositional equivalence was not demonstrated.

3.2. Relevant background data

3.2.1. Choice of comparator

Line MON 863 and the hybrid MON 863 x MON 810 were compared with control lines that had not been genetically modified and with commercial hybrids. Both F_1 generations for the MON 863 and the MON 863 x MON 810 have a similar genetic background, except for the respective inserts. These MON 863 and MON 863 x MON 810 maize hybrids were used for the studies and their self-pollination produced the respective F_2 seed generations, which were the grain material tested.

3.2.2. Agronomic Traits

One member state questioned observed morphological differences in agronomic data collected for MON 863. The agronomic parameters of six hybrid lines of MON 863 and the corresponding hybrids derived from crosses with the parental control line were compared. This included, for example, time pollen shed, plant height, and kernel moisture. Three of these hybrid lines were derived from crosses with either parental positive isolines or parental negative isolines, while the other three lines were converted inbred lines. While some statistically significant differences were noted in the comparisons of the individual hybrids of MON 863 with its control, these differences

were minor and inconsistent in that they were not observed in other transgenic hybrids. The overall average measurements did not display statistically significant differences and therefore the Panel does not consider that further agronomic data are required.

Regarding the MON 863 x MON 810 hybrid, 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 hybrid did not show any agronomic differences. The panel accepts the absence of further agronomic data for the maize hybrid.

3.2.3. Compositional analysis

Some Member States questioned the adequacy of the compositional analyses undertaken. Compositional analyses of MON 863 hybrids were carried out on kernels obtained from field trials in the United States and Argentina. In both cases 4 locations with replications in each location were used. These geographical regions are representative of areas that export maize kernels to the EU. The data included macro-nutrients, micro-nutrients, and anti-nutrients, as well as secondary metabolites in one season. Cultivated maize lines included MON 863, a non-transgenic control (MON 846) and commercial lines. In the comparisons for each separate location and all locations together, a statistically significant difference was observed for palmitic acid between MON 863 and its control. However, this difference is small and within the historical background range.

One Member State questioned compositional data for copper. The comparison between MON 863 and the hybrid MON 863 x MON 810 showed statistically significant difference in the copper content between these two lines with the MON 863 having the highest values. The comparison between MON 810 and MON 863 x MON 810 showed a statistically significant difference between these two lines with MON 810 having the lowest copper concentration. The copper content of the hybrid was between the values of MON 863 and MON 810 (average values were 1.98, 2.29 and 1.38 mg/kg dw respectively) and was consistent in all the field trials. The Panel does not find this unexpected given the distinct genetic backgrounds of the inbred single insert plants and the hybrid. Furthermore, the measurements of copper in commercial lines showed both high and low values compared to the transgenic lines. Therefore the Panel considers that there is no need for further assessment of the copper concentrations of the hybrid MON 863 x MON 810.

3.2.4. Conclusion

In the compositional analyses of MON 863 macro-nutrients, micro-nutrients, and anti-nutrients, as well as secondary metabolites were measured. Although some statistically significant differences were observed for palmitic acid between MON 863 and its control, these differences were small and within the historical background range and thus of no biological significance.

Comparison of MON 863, MON 810 and the hybrid MON 863 x MON 810 showed statistically significant differences in the copper content. The Panel considers that the copper levels are within normal ranges of variation and there is no need for further assessment in the hybrid MON 863 x MON 810.

4. Food/Feed Safety Assessment

4.1. Issues raised by the Member States

Directive 2001/18/EC

Variations in the levels of Cry proteins detected in the field trials for the single insert lines MON 863 and MON 810 and the hybrid MON 863 x MON 810 were questioned. The use of transgenic proteins expressed in *E. coli* was questioned with a specific concern about post-translational modification and possible stabilizing effects. The concentration of protein used in the acute toxicology tests was questioned. There was a concern about the adequacy of the assessment of allergenic potential and a request was made for the adoption of a more comprehensive approach to toxicity and allergenicity testing. The lack of toxicology data for the hybrid MON 863 x MON 810 was considered to be a serious shortcoming. There was concern about some significant differences in haematology, clinical biochemistry, urinary chemistry and organ weight in data from sub-chronic toxicity tests for MON 863. The data obtained in feeding studies with rats and chickens were considered inappropriate for the assessment of effects in ruminants, layers and pigs.

Regulation (EC) 258/97

In addition to the points raised above the allergenicity assessment was considered inadequate especially with respect to the use of *E. coli* derived Cry proteins and data for the hybrid MON 863 x MON 810. The inadequacy of extrapolation from the two single events to the hybrid MON 863 x MON 810 was stressed again with a request for a subchronic toxicity study in rats and a nutritional equivalence study in chickens. The resistance of a 59Kd fragment of Cry3Bb1 in simulated intestinal fluid was of concern with respect to allergenicity.

4.2. Relevant background data

4.2.1. Toxicology

Safety of expressed novel proteins in MON 863 and MON 863 x MON 810

Toxicology testing of Cry proteins expressed in transgenic plants often relies on the use of recombinant *E. coli* strains so as to produce a sufficient quantity of purified protein. In this case it is important to demonstrate that the plant and *E. coli* produced proteins are equivalent. For MON 863, this was questioned by one Member State.

The physicochemical and functional properties of the Cry3Bb1 variant protein were characterised using SDS-PAGE analysis, immunoblot analysis, mass spectrometric analysis, N-terminal sequencing, amino acid composition analysis, glycosylation analysis and insect bioassays. These studies were also used to determine the equivalence of Cry3Bb1 protein from MON 863 maize and Cry3Bb1 protein expressed in recombinant *E. coli*. The *E. coli* derived protein was identical in amino acid sequence to Cry3Bb1 produced in MON 863, including the unintended glutamine to arginine change that occurred as a result of mutation during the plant transformation process. These data demonstrated that *E. coli* and maize produced Cry3Bb1 proteins were physicochemically and functionally equivalent.

The only detected biochemical difference between these two proteins was the acetylation of the alanine residue at position 2 of the MON 863 maize produced protein. It has been argued that N-terminal acetylation could stabilize proteins. However the N-terminal portion of the Cry3Bb1 protein has been shown to be sensitive to degradation by proteases. In this case, the N-terminal sequence analysis indicates that both *E. coli* and MON 863 Cry3Bb1 have truncated N-termini. This difference is thus not expected to affect the outcome of the toxicity studies.

The Panel concludes that the documentation examined provide sufficient data on the equivalence of Cry3Bb1 in MON 863 and Cry3Bb1 produced by recombinant *E. coli*. Thus the *E. coli* produced Cry3Bb1 protein is acceptable as an alternative to plant derived Cry3Bb1 for use in the toxicological testing studies. In addition the purity of *E. coli* produced protein is often much higher than the plant-produced product (in this case: 92,6% and 53,9% respectively). In the case of plant-derived material, the presence of a large amount of unknown concentrated extraction product would be a serious disadvantage for toxicological testing. Using *E. coli* derived protein adequate acute toxicity data were provided for Cry3Bb1 and NptII.

One Member State questioned why different concentrations of the *E. coli* produced proteins were used for the acute toxicity studies (i.e. NptII: 100-1000-5000 mg protein per kg body weight, Cry3Bb1: 400-1100-3200 mg protein per kg body weight and Cry1Ab: 400-1000-4000 mg protein per kg body weight.) There is an international consensus that 5000 mg protein per kg animal body weight (BW) is an upper limit dose for acute toxicity testing. It is not always possible to perform feeding by gavage of test animals with such a high dose of protein, for example because of solubility. In the performed studies it was necessary to feed the animals with the highest dose of given protein in two portions with 3 or 4 hour intervals. The Panel considers that it is acceptable to choose the highest practical dose of protein in order to establish NOEL.

One Member State was concerned that the expression levels for the Cry3Bb1 protein were different in field trials in the USA and Argentina. Also, differences were observed from Argentinean field trials when comparing the concentration of Cry3Bb1 in MON 863 with MON 863 x MON 810 and Cry1Ab in MON 810 with MON 863 x MON 810. 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, as well as a non-transgenic maize MON 846 with the same genetic background. 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. The ranges of individual values were broad and showed overlap between the double- and single-trait hybrids. This reflects variability in gene expression, which may have been influenced, for example, by environmental factors not related to the genetic modification. In addition it is conceivable that heterosis in the hybrid genetic background 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 maize MON 863 x MON 810 and MON 863.

4.2.2. Safety of the whole GM food/feed

One Member State questioned the adequacy of whole food/feed animal testing data. Maize lines MON 863 and MON 810 were separately tested for toxicity as part of the diet for rats in 90-day studies. Other groups of rats within these experiments received diets containing maize from either control parental maize lines or 6 reference commercial lines. All maize lines were included in the diets at the 33% level, while

transgenic and control diets were also included at the 11% level (and supplemented with other non-transgenic maize to 33%). Analysis was performed on feed consumption, body weight, clinically observable adverse effects, clinical pathology during life, as well as organ weights and histopathology after study termination.

Some differences were observed in haematological parameters, including total white blood cell, lymphocyte and basophil counts. White blood cell counts were slightly increased for the male test group (33% of MON 863 maize) compared to the counts of the control and reference groups. These differences appear to be due to a slight increase in the lymphocyte count and no other changes were observed in other leucocyte counts. These differences are not considered to be biologically meaningful since they fall within the standard deviation of the reference control population .

At study termination, statistically significant differences were observed for reticulocyte counts between the female animals fed 33% MON 863 and those fed the control and reference lines. Both absolute and relative reticulocyte counts were lower, but fell largely within the range of the control and reference groups. No other differences in haematological parameters were noted for MON 863 diet compared to both control and references.

Individual kidney weights of males rats fed with the 33% MON 863 diet were statistically significantly lower compared to those of animals on control diets, but fell within the mean ± 2SD for the reference control population, and are thus not considered to be biologically meaningful since they fall within normal variation. The overall conclusion is that no differences in relation to feeding in MON 863 maize were observed on kidney weights, kidney weights relative to body weights and kidney weights relative to brain weights. The high standard deviation within experimental groups is representative for both control and test groups and there were no statistically significant differences between the groups.

Analysis of microscopic pathology data of a large number of organs and tissues showed no statistically significant differences between test and control groups. However, a statistically significant lower incidence of mineralized kidney tubules was noted for rats fed 33% MON 863 maize compared to those fed the control maize during histopathology after termination. These findings are not considered to pose concerns over the safety of MON 863 maize. Kidney tubular mineralization was observed only in females and the microscopic findings in both test and control animals were of minimal grade severity. Reported microscopic changes are considered as incidental findings and not treatment related.

For rats fed 33% MON 810 maize, a statistically significantly lower albumin/globulin count was observed compared with control and overall reference lines at study termination. Rats fed on one reference line showed similar values as for those fed MON 810. These data did not raise further safety concerns over the safety of MON 810 versus conventional maize. Slightly lower values for these parameters are not considered to be related to MON 810 maize feeding given the small magnitude of the observed changes.

The results of these 90-day rodent studies do not indicate adverse effects from consumption of maize lines MON 863 and MON 810.

Member State comments include a request for a more comprehensive toxicological assessment. For MON 863 and MON 810 the Panel is of the opinion that the dossier contains well-performed toxicological studies with the relevant species of animals and a statistically well-designed set-up. These studies were performed under quality assurance

programs and OECD guidelines. Some Member States have expressed concerns that there is less comprehensive data for the hybrid MON 863 x MON 810.

Extensive data on the two single insert lines MON 863 and MON 810 have been provided, with respect to molecular characterisation, compositional analysis and food/feed safety testing. 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 the MON 863 x MON 810 hybrid. 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, the Panel was divided on the need for an additional 90-day rat study with the MON 863 x MON 810 hybrid in order to complete its safety assessment.

4.2.3. Allergenicity

The strategies in assessing the allergenic risk concentrate on 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 (EC, 2003; CAC, 2003).

Allergenicity of newly expressed proteins Cry1Ab and Cry3Bb1

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

Allergenicity of the whole plant

Another issue is that allergenicity of the whole crop could be increased as an unintended effect of the random insertion of the transgene in the genome of the host, for example through qualitative or quantitative modification of the pattern of expression of endogenous proteins. Such unintended effects may occur at each genetic modification (i.e. in MON 810 and in MON 863) but also in the double transgenic plant after crossbreeding of MON 810 and MON 863. 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.

One Member State asked for *in vivo* studies, such as investigation of protein detection in the blood of test animals, to exclude the possibility of Cry3Bb1 protein having any

allergenic impact. 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 MON 863 Cry3Bb1 protein is not stable to digestion in simulated gastric fluid and thus there is no reason to be concerned about its absorption.

4.2.4. 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.

In addition, the Panel is aware of a scientific paper that describes a feeding study with dairy cattle (Grant et al. 2003). Test diets contained 26.7% maize kernels from MON 863 maize, while a control diet with parental maize lines and two additional diets with commercial maize lines were also included. Feeding was carried out over 21 days. No effect was noted on feed consumption, body weight, milk production, milk composition, and somatic cell count.

The Panel considers that this data is 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.

4.2.5. Conclusion

Evidence is provided that there is no acute toxicity from the Cry3Bb1, Cry1Ab and NptII proteins. The GMO Panel is satisfied that the equivalence of Cry3Bb1 in MON 863 and Cry3Bb1 produced by recombinant *E. coli* was established. 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 of individual values were broad and showed overlap between the double- and single-trait hybrids, reflecting 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 maize MON 863 x MON 810 and MON 863.

The results of 90-day sub-chronic rodent studies do not indicate adverse effects from consumption of maize lines MON 863 and MON 810 and the Panel concludes that there are no resultant concerns over their safety. For these single insert lines, 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 and MON 810 support the safety evaluation of the hybrid. However, the Panel was divided on the need for additional data on the MON 863 x MON 810 hybrid itself, in particular a 90-day sub-chronic rat study with maize expressing both Cry proteins in order to complete its safety assessment.

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 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, 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 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

Concerns were raised that a detailed monitoring plan was required and that a more proactive engagement of end users was needed to monitor any observed effects. In addition there was a need to address unintended release and more research on the effect of Cry proteins on non-target species.

5.2. Relevant background data

The notification C/DE/02/09 for maize MON 863 and MON 863 x MON 810 under Directive 2001/18/EC is for import and processing only, and thus there is no requirement for scientific information on environmental effects associated with the cultivation. Maize is highly domesticated and not generally able to survive in the environment without cultivation. Maize plants are not winter hardy, they have lost their ability to release seeds from the cob and they do not occur outside cultivated land in Europe, despite cultivation for many years. In addition, there are no cross compatible wild relatives in Europe, and gene flow via pollen is largely restricted to neighbouring crops. Maize is a hybrid crop and thus imported seeds will be a segregated F2 generation and not as fit as the F1. Studies in Europe and elsewhere with MON 863 and MON 863 x MON 810 have shown no enhanced weediness or fitness. The environmental risk assessment concludes that the likelihood of unintended environmental effects due to the establishment and spread of MON 863 and MON 863 x MON 810 maize will be no different to that of traditionally bred maize. The Panel agrees with this assessment.

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. Data supplied by the applicant and other literature suggests that most protein would be denatured by enzymic activity in the intestinal tract so that little Cry toxin would survive to pass out in faeces. There would subsequently be further degradation of proteins in the manure due to microbial processes. Thus amounts of Cry proteins being distributed onto land in manure would be very low minimizing the possibility for exposure of potentially sensitive non-target organisms (e.g. soil 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.

The objectives of a monitoring plan according to Annex VII of Directive 2001/18/EC are to confirm that any assumption regarding the occurrence and impact of potential adverse effects of the GMO, or its use, in the environmental risk assessment are correct and to identify the occurrence of adverse effects of the GMO, or its use, on human health or the environment which were not anticipated in the environmental risk assessment. The scope of the monitoring plan provided by the applicant is in line with the intended uses for the GMO since the environmental risk assessment did not cover cultivation. The Panel advises that appropriate management systems should be in place to restrict seeds of maize MON 863 x MON 810 entering cultivation, as the latter requires specific approval under Directive 2001/18/EC. The Panel is not in a position to evaluate co-existence issues, which relate to risk management and not risk assessment.

5.3. Conclusion

The MON 863 and 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 the environmental risk assessment did not cover 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.

CONCLUSIONS AND RECOMMENDATIONS

GM maize MON 863 was developed to provide protection from corn rootworm by the introduction of a variant *Bacillus thuringiensis cry3Bb1* gene expressing an insecticidal protein. Selection of the insert involved the introduction of a linked antibiotic resistance gene *nptII*. The Panel considered data on the transformation process, the vectors used and the transgenic constructs in the genetically modified plant. A comparative analysis of agronomic traits and composition was undertaken and the safety of the introduced proteins and the whole food/feed was evaluated with respect to toxicology and allergenicity. A nutritional assessment and an environmental assessment including monitoring plans were undertaken. The Panel considered that sufficient data were provided to address all outstanding questions raised by the Member States and concluded that the placing on the market of MON 863 maize is unlikely to have an adverse effect on human and animal health or the environment in the context of its proposed use.

The GMO Panel also assessed the hybrid maize MON 863 x MON 810 which is produced by a conventional cross between inbred lines of maize MON 863 and MON 810. The maize MON 810 was evaluated previously for release under directive 90/220/EEC (SCP, 1998b) and for use of processed food and food ingredients (ACNFP, 1996). GM maize MON 810 provides protection from lepidopteran insects by the introduction of a *Bacillus thuringiensis cry1Ab* gene. In assessing the MON 863 x MON 810 hybrid, both the single insert lines and the hybrid were considered and data as described above for MON 863 was evaluated.

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 the MON 863 x MON 810

hybrid. However the Panel was divided over the need for confirmatory data for the risk assessment of the hybrid, in particular the need for an additional 90-day rat study with MON 863 x MON 810. Therefore the Panel could not reach agreement on the safety evaluation of the hybrid.

DOCUMENTATION PROVIDED TO EFSA

With regard to the application under Regulation 258/97:

1. Letter to Mr. Podger, dated 9 December 2003 with ref. SANCO/D/4 – D/440641 – AN/mg, from Mrs Paola Testori Coggi from the Health & Consumer Protection Directorate-General requesting a consultation of the Scientific Panel on Genetically Modified Organisms.
2. Application under Regulation (EC) N° 258/97 concerning novel foods and novel food ingredients to Robert Koch Institut, Zentrum Gentechnologie Wollankerstrasse 15, D-13187 Berlin for grains and grain derived food ingredients from insect-protected maize line MON 863 and Maize hybrid MON 863 X MON 810 (Monsanto).
3. Initial assessment report by the Robert Koch Institute (8 April 2003) – Insect-resistant maize MON 863 and MON 863 X MON 810.
4. (Erstprüfbericht des Robert Koch-Institutes (8. April 2003) Insektenresistenter Mais MON 863 und MON 863 X MON 810)
5. Member States' comments/objections.
6. Reaction by Monsanto to the Member States' comments/objections.
7. Letters from EFSA to applicant with request for clarification/additional information (ref. SR/ (2004) 003, 7 January 2004; SR/ (2004) 155, 13 February 2004).
8. Additional information from Monsanto to the GMO Panel following requests from EFSA for additional information (submitted through the German Competent Authority by email on 15 January and 27 February 2004).

With regard to the application under Directive 2001/18/EC:

1. Note to Ms. Husu-Kallio (DG SANCO), dated 10 October 2003 with ref. C4 KT D(03) 441562, from Catherine Day (DG ENVIRONMENT) concerning Notifications under Directive 2001/18/EC - Advance copy of request to EFSA concerning notification C/DE/02/9 (MON 810 X MON 863 hybrid maize).
2. Note to Mr. Podger, dated 12 November 2003 with ref. C4 KT/ D(03) 441715 from Mr. J. Delbeke concerning Notification C/DE/02/9 (MON 863 and MON 863 x MON 810), under Directive 2001/18/EC – transmission of Member State Objections to EFSA.
3. Initial comments from the Member States with regard to Notification MON 810 X MON 863 hybrid maize (Directive 2001/18/EC).
4. Meeting record between the competent authorities, applicant and Commission, on 21 October 2003, where the objections were discussed.
5. Objections from Member States with regard to Notification C/DE/02/9 (MON 810 X MON 863 hybrid maize).
6. Submission from Monsanto Services International (24 October 2003) to EFSA regarding the scientific review by EFSA of the Application for consent to place on the

market insect-protected maize MON 863 and MON 863 x MON 810 for use as any other maize, excluding marketing of varieties in the European Union (C/DE/02/9) containing

a. Notification letter for maize MON 863 and MON 863 x MON 810 (C/DE/02/9)

b. Directive 90/220/EEC application dossier for MON 863 and MON 863 x MON 810 maize including Appendices II and V

c. Corrigendum to the Directive 2001/18/EC application dossier

d. Initial assessment report by the German lead competent state

e. Response to MS questions (non-confidential part; also confidential appendices)

7. Letters from EFSA to applicant with request for clarification/additional information (ref. SR/ (2004) 003, 7 January 2004; SR/ (2004) 155, 13 February 2004).

8. Additional information from Monsanto to the GMO Panel following requests from EFSA for additional information (submitted through the German Competent Authority by email on 15 January and 27 February 2004).

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

Adams, K.L., Daley, D.O., Qiu, Y.-L., Whelan, J. and Palmer, J.D., 2000. Repeated, recent and diverse transfers of a mitochondrial gene to the nucleus in flowering plants. Nature, 408, 354-357.

Adams, K.L., Qui, Y.L., Stoutemyer, M. and Palmer, J.D., 2002. Punctuated evolution of mitochondrial gene content: high and variable rates of mitochondrial gene loss and transfer to the nucleus during angiosperm evolution. Proc. Natl. Acad. Sci. USA, 99, 9905-9912.

Adams, K.L., Song, K., Roessler, P.G., Nugent, J.M., Doyle, J.L., Doyle, J.J. and Palmer, J.D., 1999. Intracellular gene transfer in action: dual transcription and multiple silencings of nuclear and mitochondrial *cox*2 genes in legumes. Proc. Natl. Acad. Sci. USA, 96, 13863-13868.

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

Blanchard, J.L. and Schmidt, G.W., 1995. Pervasive migration of organellar DNA to the nucleus in plants. J. Mol. Evol. 41, 397-406.

Braun, H.P., Jänsch, L., Kruft, V. and Schmitz, U.K., 1994. The 'Hinge' protein of cytochrome *c* reductase from potato lacks the acidic domain and has no cleavable presequence. FEBS Lett., 347, 90-94.

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.

Daley, D.O., Adams, K.L., Clifton, R., Qualmann, S., Millar, A.H., Palmer, J.D., Pratje, E. and Whelan, J., 2002. Gene transfer from mitochondrion to nucleus: novel mechanisms for gene activation from Cox2. Plant J., 30, 11-21.

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

EC, 1997. Regulation (EC) No 258/97 of the European Parliament and of the Council of 27 January 1997 concerning novel foods and novel food ingredients. 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, 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.pdfb

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

EC, 2003. Guidance document for the risk assessment of genetically modified plants and derived food and feed, prepared by the Joint Working Group on Novel Foods and GMOs, 6-7 March 2003. http://europa.eu.int/comm/food/fs/sc/ssc/out327_en.pdf

EFSA, 2003. Opinion of the Scientific Panel on Genetically Modified Organisms on a request from the Commission related to the Notification (Reference CE/ES/00/01) for the placing on the market of herbicide-tolerant genetically modified maize NK603, for import and processing, under Part C of Directive 2001/18/EC from Monsanto. The EFSA Journal, 10, 1-13. http://www.efsa.eu.int/pdf/gmo/opinion_gmo_03_final_en.pdf

EFSA, 2004. Opinion of the Scientific Panel on Genetically Modified Organisms on the use of antibiotic resistance genes as marker genes in genetically modified plants. The EFSA Journal, 48, 1-18. http://www.efsa.eu.int/science/gmo/gmo_opinions/384_en.html

Figueroa, P., Gomez, I., Carmona, R., Holuigue, L., Araya, A. and Jordana, X., 1999a. The gene for mitochondrial ribosomal protein S14 has been transferred to the nucleus in *Arabidopsis thaliana*. Mol. Gen. Genet., 262, 139-144.

Figueroa, P., Gomez, I., Holuigue, L., Araya, A. and Jordana, X., 1999b. Transfer of *rps*14 from the mitochondrion to the nucleus in maize implied integration within a gene encoding the iron-sulphur subunit of succinate dehydrogenase and expression by alternative splicing. Plant J., 18, 601-609.

Fischer, R.L., Miller , P.S., Hyun, Y., Hartnell, F.F. and Stanisiewski, E.P., 2003. Comparison of swine performance when fed diets containing corn root worm protected corn, parental line corn, or conventional corn grown during 2000 in Nebraska. J. Anim. Sci., 81, 207-.

Fukuchi, M., Shikanai, T., Kossykh, V.G. and Yamada, Y., 1991. Analysis of nuclear sequences homologous to the B4 plasmid-like DNA of rice mitochondria; evidence for sequence transfer from mitochondria to nuclei. Curr. Genet., 20, 487-494.

Goff, S.A., Ricke, D., Lan, T.H., Presting, G., Wang, R., Dunn, M. and etc., 2002. A draft sequence of the rice genome *(Oryza sativa* L. ssp. *japonica*). Science, 296, 92-100.

Grant, R.J ., Fanning, K.C., Kleinschmit , D., Stanisiewski, E.P. and Hartnell, G.F., 2003. Influence of glyphosate-tolerant (event NK603) and corn root worm protected (event MON 863) corn silage and grain on feed consumption and milk production in Holstein cattle. J. Dairy Sci., 86, 1707-1715.

Holck,A., Vaïtilingom,M., Didierjean,L. and Rudi,K., 2002. 5'-Nuclease PCR for quantitative event-specific detection of the genetically modified MON 810 MaisGard maize. Eur. Food Res. Technol., 214, 449–453.

Kemble, R.J., Mans, R.J., Gabay-Laughnan, S. and Laughnan, J.R., 1983. Sequences homologous to episomal mitochondrial DNAs in the maize nuclear genome. Nature 304, 744-747.

Kubo, N., Takano, M., Nishiguchi, M. and Kadowaki, K.I., 2001. Mitochondrial sequence migrated downstream to a nuclear V-ATPase B gene is transcribed but non-functional. Gene 271, 193-201

Lam, E. and Chua, N., 1990. GT-1 binding site confers light responsive expression in transgenic tobacco. Sci., 248, 471-474.

Mackenzie, S. and McIntosh, L., 1999. Higher plant mitochondria. Plant Cell 11, 571-585.

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.

Nap, J.P., Bijvoet, J. and Strikema, W.J., 1992. Biosafety of kanamycin-resistant transgenic plants: an overview. Transgenic Crops, 1, 239-249.

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.

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, 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

Sun, C.W. and Callis, J., 1993. Recent stable insertion of mitochondrial DNA into an Arabidopsis polyubiquitin gene by nonhomologous recombination. Plant Cell 5, 97-107.

Vander Pol, K., Simon, J., Erickson, G., Klopfenstein, T., Stanisiewski, E. and Hartnell G., 2003. Feeding transgenic (Bt corn rootworm protected and Roundup-Ready) corn to feedlot cattle. 2003 Nebraska Beef Report, 30-32.

Wilson, C.B., Macken, C.N., Er ickson, G.E., Klopfenstein, T.J . and Stanisiewski, E., 2003. Utilization of genetically enhanced corn residue on grazing steer performance. Nebraska Beef Report , 18-19.

SCIENTIFIC PANEL MEMBERS[6]

Hans Christer Andersson, Detlef Bartsch, Hans-Joerg Buhk, Howard Davies, Marc De Loose, Michael Gasson, Niels Hendriksen, Colin Hill, 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 Folmer Eriksen and Gijs Kleter for their contributions to the draft opinion.

[6] Information on the declarations of interest can be found in the minutes of the 5th Plenary meeting of the GMO Panel: http://www.efsa.eu.int/pdf/minutes_gmo_05_final_en.pdf