

**ASSESSMENT REPORT OF THE UK COMPETENT AUTHORITY IN  
ACCORDANCE WITH DIRECTIVE 2001/18/EC**

**NOTIFICATION C/GB/02/M3/3 FROM MONSANTO EUROPE S.A. FOR  
CONSENT TO MARKET HYBRID MAIZE (NK603 X MON810)**

**5 MARCH 2004**

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## **1. Introduction**

Monsanto Europe S.A. (Monsanto) has developed hybrid maize (*Zea mays* L.) varieties that are produced by traditional breeding, of genetically modified maize inbred parental lines derived from maize transformation events NK603 and MON810. The resultant hybrid maize, to which this notification applies, is known as NK603 X MON810. The product has been designated a unique identifier MON-ØØ6Ø3-6 × MON-ØØ81Ø-6. The hybrid seed used for planting is marketed under the name of the hybrid variety, in association with the trademarks Roundup Ready® and YieldGard®. The transformation event NK603 confers tolerance to the herbicide Roundup (containing glyphosate) and the transformation event MON810 confers resistance to larvae of specific lepidopteran insect pests. NK603 X MON810 hybrid maize has been commercially grown in the USA since 2002.

Line MON810 was approved for cultivation and importation in Europe in 1998 (consent reference: C/F/95/12-02). Line MON810 has also been considered as a parental line of MON863 X MON810 maize hybrid. This dossier came forward from the German authorities (reference C/DE/02/9), the consent for importation is still pending. Line NK603 has also been considered in a notification forwarded from the Spanish Competent Authorities (reference C/ES/00/01), the consent for importation is still pending.

### **1.1. Procedure**

The UK competent authority accepted this dossier on 17 April 2002 under Directive 90/220/EC. Under the provisions of Article 35 of Directive 2001/18/EC on pending notifications, the UK CA accepted this dossier on 8 December 2002. The dossier has been assessed with reference to Article 13(2) of Directive 2001/18/EC. During the assessment period further information was requested from the applicant as follows:

➤ *Information on the molecular characterisation of both parental transformation events*

Experimental data were requested to provide: (1) evidence that the copy number of the inserts was one; (2) evidence that the vector backbone sequences were absent; (3) to provide DNA sequence data flanking the insertion sites. These were satisfactorily provided.

Clock stopped: 08/12/02 to 27/06/03 and 08/07/03 to 17/12/03.

➤ *Information on comparative compositional analysis*

Further data were requested: (1) to substantiate the claim that changes in phosphorus levels in the hybrid GM maize were not significantly different to its non-GM counterpart; (2) a statistical meta analysis of compositional data from the four EU trial sites for the NK603 parental line in order to support the conclusion that NK603 X MON810 maize is nutritionally equivalent to its non-transgenic counterpart and therefore provide further support for earlier conclusions regarding the safety of NK603 X MON810 hybrid maize grain.

These were satisfactorily provided.

Clock stopped: 08/12/02 to 27/06/03 and 08/07/03 to 17/12/03.

➤ *Information on animal feeding studies*

Provision of full data on both rat and broiler chicken feeding experiments on parental line NK603 were requested in order to make a full assessment of the safety of NK603 X MON810 as animal feed. These data were satisfactorily provided.

Clock stopped: 08/12/02 to 27/06/03.

➤ *Information on potential allergenicity/toxicity to humans*

Further supporting data were requested to justify statements made regarding the low allergenicity/toxicity of NK603 X MON810 maize. These data were provided by the notifier.

Clock stopped: 08/12/02 to 27/06/03.

➤ *A revised environmental risk assessment*

The UK noted that the scope of this application is for consent to import and use (but not to cultivate) F2 grain harvested from F1 NK603 X MON810 maize in the EU. A revised environmental risk assessment was requested to include an analysis of the stability and possible recombination events during the production of F2 grain. The notifier satisfactorily provided these data.

Clock stopped: 08/12/02 to 27/06/03 and 08/07/03 to 17/12/03.

➤ *A revised post-market monitoring plan*

A revised plan was requested to take into account the additional risk assessments concerning the molecular characterisation and stability of the F2 grain requested above and to provide a more proactive surveillance monitoring programme. The notifier satisfactorily provided this information.

Clock stopped: 08/12/02 to 27/06/03 and 08/07/03 to 17/12/03.

## **1.2. Scientific advice**

Two independent scientific committees in the UK, the Advisory Committee on Releases to the Environment (ACRE) and the Advisory Committee on Animal Feedingstuffs (ACAF) have considered this notification. The advice of ACRE is provided at Annex 1 and incorporates the opinion of ACAF.

## **1.3 Public comments**

The Summary Notification Information Format (SNIF) published on the Joint Research Centre website generated 33 representations from Members of the public originating from the UK, the Netherlands, Spain, Belgium, Sweden and Portugal and Italy. Some representations were long (4-9 pages). Where necessary, the UK made arrangements for representations to be translated into English. All representations were taken into account in the UK assessment of this dossier.

## **2. List of documents**

The dossier consists of:

- Information required according to Annex III B of Directive 2001/18/EC (pp 11-64).
- Additional information according to Annex IV of Directive 2001/18/EC (pp 65-74).

- Risk assessment according to Annex II of Directive 2001/18/EC (pp 75-87).
- Monitoring plan according to Annex VII of Directive 2001/18/EC (pp 88-96).
- Appendices I - IV.

## **2.1. Confidentiality**

Monsanto claimed confidentiality for some of the further information supplied regarding aspects of additional molecular characterisation and animal feed safety assessments in order to protect the Company's business and competitiveness. This confidentiality was respected by the UK during the assessment process however in line with Article 25(4) of Directive 2001/18/EC a condition of consent will be that the event specific detection protocol for NK603 is made public (see item 10.1). The protocol for MON810 is non-confidential.

## **3. Scope of the notification**

The scope of this application is for the import of raw commodities containing maize grain derived from NK603 X MON810 and the import of processed food/feed products containing maize originating from maize grain derived from NK603 X MON810. This notification does not include cultivation in the EU.

## **4. Description of the product**

Hybrid maize NK603 X MON810 is produced by a single traditional cross of NK603 maize and MON810 maize inbred lines (homozygous for the respective introduced trait). The F1 hybrid seed is used for crop production. The harvested F2 grain is for import and use as any other maize, but not for further cultivation. The intended functions of the genetic modifications are tolerance to glyphosate (NK603) and resistance to lepidopteran larvae of *Sesamia* spp. and *Ostrinia nubilalis* (MON810).

### **4.1. Transformation technique**

NK603: The transformation event has been produced by particle bombardment, also known as acceleration technology, of maize cell culture line AW X CW with a 6.7 kb *MluI* fragment of the bacterial plasmid vector PV-ZMGT32.

MON810: The transformation event has been produced by particle bombardment of genotype Hi-II with a 5.5 kb *NdeI* fragment of the plasmid PV-ZMBK07.

### **4.2. Molecular and genetic description**

#### **4.2.1. The plasmids**

NK603: The plasmid PV-ZMGT32 contains the origin of replication sequence from *E. coli* pUC19 plasmid and the *nptII* gene of the *Tn5* transposon from *E. coli*, under the control of its own promoter. Additionally, the plasmid contains two adjacent plant gene expression cassettes, each containing one copy of a gene coding for glyphosate tolerance, 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) from *Agrobacterium* sp. strain CP4 and each cassette is fused to chloroplast transit peptide (*ctp2*) sequences based on sequences

derived from *Arabidopsis thaliana*. Expression of the first *ctp2-cp4 epsps* cassette is regulated by the rice actin promoter and a rice intron sequence introduced upstream of the *ctp2* sequence. Expression of the second *ctp2-cp4 epsps* cassette is regulated by the 35S promoter from Cauliflower Mosaic Virus (CaMV) with a double enhancer region (e35S) and a maize intron derived from a *hsp70* gene encoding a heat shock protein. Each cassette is linked to the transcription terminator, the non-translated DNA sequence nopaline synthase (*nos 3'*) sequence from *A. tumefaciens*.

MON810: The plasmid PV-ZMBK07 is a derivative of pUC19. In addition to the origin of replication and the *lac* sequences, the plasmid contains the *nptII* gene of the *Tn5* transposon from *E. coli*, under the control of its own promoter. Additionally the plasmid contains the *cry1A(b)* gene encoding  $\delta$ -endotoxin from *Bacillus thuringiensis* ssp. *kurstaki* HD1 strain. The *hsp70* gene is inserted upstream of the  $\delta$ -endotoxin coding region. Expression is controlled by the enhanced 35S promoter from CaMV (e35S). The transcription terminator used was the nopaline synthase (*nos 3'*) gene from *A. tumefaciens*.

#### **4.2.2. Genetic elements introduced into the GMO**

NK603: The transforming 6.7 kb *MluI* fragment is derived from plasmid PV-ZMGT32 and contains:

- *cp4 epsps* gene from *Agrobacterium* sp. strain CP4 (cassette 1).
- *cp4 epsps L214P* gene from *Agrobacterium* sp. strain CP4 (cassette 2)
- *ctp2* sequences derived from *Arabidopsis thaliana* (both cassettes).
- Rice actin promoter and rice intron sequence (cassette 1).
- CaMV 35S promoter and maize heat shock protein *hsp70* gene (cassette 2).
- The *nos 3'* terminator from *A. tumefaciens* (both cassettes).

No antibiotic resistance markers are present in this restriction fragment.

MON810: The transforming 5.5 kb *NdeI* fragment is derived from plasmid PV-ZMBK07 and contains:

- The *Cry1A(b)* gene from the soil microorganism *Bacillus thuringiensis*.
- CaMV 35S promoter and maize heat shock protein *hsp70* gene.

No antibiotic resistance markers are present in this restriction fragment.

#### **4.2.3. Molecular characterisation**

The molecular characterisation of NK603 X MON810 is of sufficient quality to allow the assessment of any potential hazards. Evaluation of the GM maize hybrid was carried out on the basis of information on the parental lines rather than full characterisation of the maize hybrid. The DNA fragments used in both transformation events do not carry the *nptII* gene or any other plasmid backbone sequences. Information is supplied to demonstrate that these sequences are absent from the parental lines NK603 and MON810 maize and they will, therefore, also be absent from the hybrid NK603 X MON810.

NK603: Nucleotide sequence analysis has determined that gene expression cassette 1 in the insert is identical to that of the donor plasmid vector. Sequence analysis of gene expression cassette 2 in the insert revealed two

nucleotide changes compared to the donor plasmid, therefore the gene is designated *cp4 epsps L214P*. At the 3' end, the insert includes an inversely linked 217 bp DNA fragment of the enhancer region of the rice actin promoter but does not contain sequences required for promoter activity. Southern transfer and hybridisation reveals that the NK603 transformation insert is present as a single copy in the plant genome. Sequence analysis and comparison with sequence databases has located the site of integration in event NK603 as the nuclear maize genome. It has been confirmed that the 3' and 5' ends of the insert are contained within the maize genome and flanking sequences have been identified to 307 bp 5' and 497 bp 3' of the NK603 insert. Separate Southern transfer and hybridisation experiments establish that the inserted DNA has been stably incorporated over multiple generations and when the plant is grown in different locations. The stability of insertion may also be inferred from the predicted Mendelian inheritance of the herbicide tolerance trait observed for NK603 X MON810 plants.

MON810: Nucleotide sequence analysis has determined that the inserted DNA sequence is identical to that of the donor plasmid vector. Southern transfer and hybridisation reveals that the MON810 transformation insert is present as a single copy in the plant genome. Further sequence analysis and comparison with sequence databases has located the site of integration in event MON810 as the nuclear maize genome. It has been confirmed that the 3' and 5' ends of the insert are contained within the maize genome and flanking sequences have been identified to 244 bp 5' and 606 bp 3' of the MON810 insert. Separate Southern transfer and hybridisation experiments establish that the inserted DNA has been stably incorporated over multiple generations and when the plant is grown in different locations. The stability of insertion may also be inferred from the predicted Mendelian inheritance of the herbicide tolerance trait observed for NK603 X MON810 plants.

Bioinformatic analyses of the amino acid sequences for both inserts were performed by comparing all possible open reading frames of the junction regions of the insert and the plant genome flanking regions. The data revealed that there were no immunological or structural similarities to known pharmacologically active proteins, allergens or toxins.

#### **4.2.4. Gene expression**

NK603: Expression of the *Agrobacterium epsps* gene, confers tolerance to glyphosate, the active ingredient in Roundup herbicide. EPSPS protein concentrations were determined by ELISA and were higher in forage NK603 X MON810 (average 36.3 µg/g fresh weight) than in grain (average 12.7 µg/g fresh weight). The levels of CP4 EPSPS protein in samples from each tissue type in the non-transgenic control hybrid were below the limit of detection (0.38 µg/g fw for forage and 0.08 µg/g fw for grain).

MON810: Expression of the *cry1A(b)* *B. thuringiensis* gene encodes a Bt toxin, which has insecticidal activity towards certain species of lepidopteran larvae. Cry1A(b) protein concentrations were higher in forage NK603 X MON810 (average 6.06 µg/g fresh weight) than in grain (average 0.73 µg/g fresh weight). The levels of Cry1A(b) protein in samples from each tissue type

in the non-transgenic control hybrid were below the limit of detection (0.27 µg/g fw for forage and 0.13 µg/g fw for grain).

Gene expression of both inserts occurs throughout the whole plant (e.g., roots, stem, pollen, seed, leaf).

## **5. Assessment of use in animal feeds**

The primary use of the maize grain is for animal feed, however maize grain is also processed into human food and industrial products. The ACAF GM sub-group was consulted on animal feed safety aspects.

### **5.1. Safety of gene products**

The UK recognises that the EPSPS protein encoded by the *epsps* gene from *Agrobacterium* sp. strain CP4 has been examined on a number of previous occasions and deemed to be safe. The safety of the CP4 EPSPS protein from the NK603 line was demonstrated through evidence of the proteins susceptibility to proteases in simulated gastric and intestinal models as well as an absence of adverse responses when the EPSPS product was used in an acute mouse toxicity study. Equivalent studies and safety assessments were made with the Cry1A(b) protein in pursuit of approval for line MON810. The experimental details provide adequate reassurance of the lack of toxicity of the *epsps* and *Cry1A(b)* gene products.

### **5.2. Compositional analysis**

Compositional analysis was conducted on NK603 X MON810 hybrid maize grain and forage samples taken in 3 one-year field trial sites in France compared with samples from a non-transgenic control hybrid and 5 different non-transgenic commercial maize hybrids grown in replicated plots at the same field sites. Raw grains were analysed for their proximate composition, amino acid, fatty acid, fibre, secondary metabolite, mineral and vitamin content and for a number of anti-nutritional factors. Although for a number of nutrients significant differences were found, including phosphorus levels, the range of the values fell within the range reported for the non-GM test hybrid.

There is nothing in the body of compositional data to suggest that NK603 X MON810 hybrid maize differs in any biologically significant way from that of the control varieties and it can be concluded that it is compositionally equivalent to its non-transgenic control hybrid line and to other commercial hybrids.

### **5.3. Feeding studies**

NK603: A study to evaluate the nutritional value of NK603 maize for poultry was made over a 42 day period. The broiler chick feeding study comprised of a total of seven treatments (NK603, the corresponding parental control and five representative reference maize lines). These were assigned to five blocks in a randomised complete block design. No differences were observed in the growth of birds fed NK603 maize compared to the parental control or other reference maize lines. Neither was there evidence of any unintended effects, the results therefore confirm the conclusion that event NK603 is nutritionally equivalent to its non-transgenic counterpart.

A rat feeding study incorporated maize line NK603 into rat diets and fed to a group of 40 rats (20 male and 20 female) for a period of 13 weeks. Weekly feed consumption and weight gain and various clinical and haematological parameters were measured and compared to the equivalent results from rats fed the same amount of a non-GM parental hybrid or one of a number of reference hybrids. All rats were killed at the end of the study period, organ weights were recorded and tissue samples were collected for microscopic examination. There were occasional observations of differences in weight gain between rats fed NK603 and the parental control groups. However, where such differences were recorded, they occurred within one sex, at one intermediate observation point and at one dose level, and the weights still fell within the range observed in rats fed the reference maize diets. Similarly for the majority of haematological and clinical chemical parameters measured there were no statistically significant differences reported between control and NK603 groups. Where differences were observed, these did not appear dose related and again generally did not fall outside the range established for the reference diets. These observations also support a view that NK603 behaves as any other maize kernels and that no unintended effects introduced by the event that might compromise the safety of this maize line were present.

MON810: Experimental details of rat and broiler chick feeding studies, generated after the original EU approval was granted for MON810 maize (notification C/F/95/12-02), were provided. These additional data add weight to the original conclusion that led to market approval, that MON810 behaves as any other maize variety.

NK603 X MON 810: Poultry feeding studies were conducted to demonstrate the safety of NK603 X MON810 maize grain as animal feed. A 42-day broiler chicken feeding study was undertaken in which NK603 X MON810 maize grain was compared with the non-transgenic control maize and six commercial hybrids in a randomised complete block design. Weight gain was not statistically different between treatments. For most of the carcass measurements undertaken, no statistically significant differences were observed between broilers fed on the NK603 X MON810 grain and the control or commercial strains. The protein content of the breast meat of the broilers fed the transgenic maize was lower than that of broilers fed the control maize and one of the commercial maize lines, but was not different from the other commercial lines. The results of this study confirm that hybrid maize NK603 X MON810 is nutritionally equivalent to its non-transgenic counterpart and therefore provide further support for earlier conclusions regarding the safety of NK603 and MON810 maize grain.

In summary, the above animal feeding data support the view that the kernels from the parental maize lines (NK603 and MON810) and NK603 X MON810 hybrid maize behave as any other maize when used in the diets of animals.

#### **5.4. Conclusion**

Overall, the compositional equivalence of NK603 X MON810 grain, together with the results of the rat and poultry studies, suggests that grain from the

parental lines and the NK603 X MON810 hybrid line would behave as any other equivalent variety of maize and would pose no greater risk to livestock or consumers of livestock products than any other commercial variety of maize.

## **6. Assessment of environmental risks**

In considering the environmental risk assessment for NK603 X MON810 hybrid maize, the UK has restricted its assessment to consider issues which are relevant within the scope with which the notification is made, that is for the import and processing of NK603 X MON810 maize grain and its use for animal feed. In respect of this, the effects of the GMO on biogeochemical processes and impacts arising from changes in management practices did not need to be considered.

The environmental risk assessment does not identify any potential differences between conventionally bred NK603 X MON810 hybrid maize and non-transgenic maize varieties for phenotypic characteristics, with the exception of the two new characteristics, tolerance to glyphosate and insect resistance.

### **6.1. Potential gene dissemination by pollen or seed**

Due to the low germination rate and subsequent low viability of any germinated maize plants there were no anticipated environmental risk problems. For the purpose of this notification F2 grain from NK603 X MON810 hybrid maize will be imported for direct use as food and feed and for processing. No seeds will be imported for cultivation into Europe.

### **6.2. Potential for gene transfer**

No differences in dissemination capacity or increased potential for gene transfer have been observed in pollen, seed and vegetative material from NK603 X MON810 compared with non-GM maize. Since maize does not establish properly outside the agricultural environment, the impact of escape of grain during storage or transport on gene transfer into other maize crops or weeds was considered to be extremely low. Due to the low germination rate and subsequent low viability of any germinated maize volunteer plants there were no anticipated environmental risk problems.

The potential for transfer of genetic material from NK603 X MON810 maize is no different to that for conventional maize varieties. Gene flow will only occur into other cultivated maize plants, however the likelihood of gene transfer is very low due to a combination of barriers most specifically the fact that this notification is not for authorisation for cultivation.

### **6.3. Safety of non-target organisms**

Within the scope of this notification, NK603 X MON810 maize has no specific effects on non-target organisms when compared to non-GM maize varieties.

## **7. Detection method**

Satisfactory PCR based protocols that are suitable for event specific detection of each parental line (NK603 and MON810) have been provided.

## **8. Surveillance and monitoring plan**

A post-market monitoring plan is provided. The conclusion of the risk assessment does not identify a requirement for any case specific monitoring. Therefore, only a monitoring program for general surveillance is proposed.

General surveillance will make use of those people and their networks that are responsible for transport, processing and handling of the GM hybrid maize grain. In order to achieve monitoring, Monsanto will ensure that those handling the GM hybrid maize grain are provided with information on the product and their duties within the supply chain. A report of the general surveillance programme will be made by Monsanto on an annual basis after the product has been approved for marketing (as a condition of consent see item 10.1). However, should any adverse effect that alters the risk assessment be identified then Monsanto will have a responsibility to immediately inform the authorities.

The post-market monitoring plan does not include a proposal to monitor for spillage of seed. The UK is content that any spilt seed (F2 grain) is not a risk due to the fact that it will have limited or no viability even within an agronomic environment.

## **9. Traceability and labelling**

Marketing of this product will be subject to the requirements of Regulation EC No 1829/2003 on traceability and labelling.

## **10. Conclusion**

Based on the information in the notification dossier the UK Competent Authority concludes that there is no reason within the scope of Directive 2001/18/EC why consent for placing this product on the EU market for the purpose of import and processing (excluding cultivation) should be withheld.

The UK therefore proposes to issue consent, subject to a number of conditions, to Monsanto Europe S.A. for the placing on the market of NK603 X MON810 hybrid maize. The proposed consent covers the import and use for all processing and use in feed of NK603 X MON810 maize. The proposed consent does not include the cultivation of NK603 X MON810 maize.

### **10.1. Conditions of consent**

The proposed consent will be granted to Monsanto Europe S.A. with the following conditions:

- The UK considers that the consent holder should be required to report on post-market monitoring as follows:
  - at least 30 days before NK603 X MON810 is placed on the market the consent holder should provide further details of the arrangements for carrying out general surveillance, including: (1) precisely who will be requested to provide information; (2) what type of information will be requested and the frequency of requests and (3) how the company will ensure participation to ensure a robust assessment.

- annually from the date that NK603 X MON810 is first placed on the market. These subsequent reports should detail the outcomes of the general surveillance monitoring.
- In line with Article 25 (4) of Directive 2001/18/EC, the discriminating PCR protocol for the NK603 insert must be made public.
- Appropriate reference material of the F1 NK603 X MON810 hybrid must be provided to the Joint Research Centre upon request.
- The consent will be valid for a period of 10 years from the date of issue.

ACRE ADVICE ON C/GB/02/M3/3

**ADVISORY COMMITTEE ON RELEASES TO  
THE ENVIRONMENT**



*Advice on a notification for marketing of  
herbicide tolerant and insect resistant GM  
hybrid maize*

**Notifier:** Monsanto Europe S.A.

**Notification  
reference:** C/GB/02/M3/3

**Product:** Hybrid maize genetically modified for herbicide tolerance and insect resistance, transformation events NK603 and MON810.

**Scope:** For the import of grain derived from hybrid maize containing events NK603 and MON810 and for processing and use as for any other maize. This notification excludes cultivation.

**Date:** 30 January 2004

**Advice of the Advisory Committee on Releases to the Environment (ACRE) under S.124 of the Environmental Protection Act 1990 (Part VI) to the Secretary of State for Environment, Food and Rural Affairs, Scottish Ministers, Ministers of the Welsh Assembly Government and the Department of Environment (Northern Ireland).**

**Advice:** ACRE has considered this notification for the import and use of herbicide tolerant and insect resistant hybrid maize based on transformation events NK603 and MON810. The Committee considers that sufficient information has been provided by the notifier to demonstrate that this hybrid GM maize does not pose a risk to human health or the environment. The marketing of this product for importation and processing in the UK will be no different from that of other maize imported for processing and animal feed purposes. In coming to this conclusion ACRE have taken account of the advice of the Advisory Committee on Animal Feedingstuffs (ACAF) Genetic Modification sub-group.

ACRE recommends that if consent is issued, it should be conditional on the notifier providing detailed arrangements for general surveillance of this product. Post-market monitoring reports should be provided to the regulatory authorities on an annual basis.

## **Comment**

This notification was received by the UK as the lead competent authority. ACRE considered this notification and the potential risks arising from importation and commercial use of this GM maize. The scope of the notification excludes cultivation and the Committee considered this notification in this context.

## **Molecular characterisation**

Hybrid maize NK603 X MON810 is produced by a single traditional cross of NK603 maize and MON810 maize inbred lines (homozygous for the respective introduced trait). The F1 hybrid seed is used for crop production. The harvested F2 grain is for import and use as any other maize, but not for further cultivation.

Evaluation of the GM maize hybrid was carried out on the basis of information on the parental lines rather than full molecular characterisation of the maize hybrid. The reader is referred to notification C/ES/00/01 for the import and use of NK603 as for any other maize and to consent C/F/95/12-02 issued in April 1998 by France for the cultivation and use of MON810 as for any other maize.

NK603: Transformation event NK603 has been produced by particle bombardment of a maize cell culture line with a 6.7 kb *Mlu*I fragment of the bacterial plasmid vector PV-ZMGT32. This DNA fragment includes two plant gene expression cassettes, each containing a copy of a gene coding for glyphosate tolerance, 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) from *Agrobacterium* sp. strain CP4 engineered to be under the control of the rice actin promoter (cassette 1) and the enhanced Cauliflower Mosaic Virus 35S (*e35S*) promoter (cassette 2) and the nopaline synthase terminator (NOS) sequence. Expression of the *Agrobacterium* CP4 *epsps* gene confers tolerance to glyphosate, the active ingredient in Roundup herbicide.

ACRE considered that the molecular characterisation of event NK603 was of sufficient quality to allow the assessment of any potential hazards. The Committee is content that the data support the conclusion that the insert is present as a single copy in the nuclear maize genome and that vector backbone sequences are absent from maize line NK603. Sequence analysis concludes that gene expression cassette 1 in the insert is identical to that of the donor plasmid. Sequence analysis of gene expression cassette 2 in the insert revealed two nucleotide changes compared to the donor plasmid and at the 3' end, the insert includes an inversely linked 217 bp DNA fragment of the enhancer region of the rice actin promoter but does not contain sequences required for promoter activity. ACRE considered that these molecular rearrangements did not pose any safety risks. ACRE is satisfied that the 3' and 5' ends of the insert are contained within the maize genome and with the identification of the flanking region sequences to 307 bp 5' and 497 bp 3' of the NK603 insert. A short RNA (1.4 kb) transcription product was detected which initiated from the 3' end of the NK603 insert and extended beyond the NOS 3' terminator into the maize genome flanking the 3' end of the inserted DNA. The committee is content that that this short "read through" transcription product is not expected to have a regulatory function and that it does not alter

the risk assessment for NK603 maize. In addition, ACRE is content that the PCR detection protocol provided for NK603 is event-specific.

MON810: Transformation event MON810 has been produced by particle bombardment of maize with a 5.5 kb *Nde*I fragment of the plasmid PV-ZMBK07, which contains one copy of the *e35S* promoter, the maize heat shock protein gene (*hsp70*) intron and most of the *Cry1A(b)* open reading frame, sufficient to encode the insecticidally active *Cry1A(b)* protein.

ACRE considered the molecular characterisation for event MON810 was of sufficient quality to allow the assessment of any potential hazards. The Committee is content that the data support the conclusion that the inserted DNA sequences are present as a single copy in the nuclear maize genome and that vector backbone sequences are absent from the maize line MON810. Sequence analysis concludes that the insert is identical to that of the donor plasmid. ACRE is satisfied that the 3' and 5' ends of the insert are contained within the maize genome and with the identification of the flanking region sequences to 244 bp 5' and 606 bp 3' of the MON810 insert. The committee is satisfied that the molecular characterisation of MON810 does not indicate any potential hazards. In addition, ACRE is content that the PCR detection protocol provided for MON810 is event-specific.

### **Animal feed safety**

The safety of grain derived from NK603 X MON810 maize for use as animal feed was assessed by the ACAF GM sub-group. In considering the safety of NK603 X MON810 for use in animal feed, the safety of the gene products, compositional analysis and data from animal feeding studies were taken into account.

### Safety of the expressed proteins

There is a growing and substantial body of data on the CP4 EPSPS protein establishing its inherent safety in other maize lines and in other crops. ACAF is content that there is sufficient evidence to demonstrate the safety of the CP4 EPSPS protein from the NK603 line. Equivalent studies and safety assessments were made with the *Cry1A(b)* protein in pursuit of approval for line MON810.

### Comparative compositional analysis

Compositional analysis was conducted on NK603 X MON810 hybrid maize grain and forage samples taken in 3 one-year field trial sites in France compared with samples from a non-transgenic control hybrid and 5 different non-transgenic commercial maize hybrids grown in replicated plots at the same field sites. Raw grains were analysed for their proximate composition, amino acid and fatty acid content and for a number of anti-nutritional factors. ACAF is content that NK603 X MON810 hybrid maize does not differ in any biologically significant way from that of the control varieties and that it is compositionally equivalent to its non-transgenic control hybrid line and to other commercial hybrids.

### Animal feeding studies

**NK603**: The data provided on rat and broiler chicken feeding experiments on parental line NK603 demonstrate that no significant differences were

observed in the growth of broiler chickens or rats fed NK603 maize compared to the parental non-transgenic control or other reference maize lines. Therefore, NK603 maize delivered the nutrition expected from the compositional analysis. ACAF is content that the results support a view that NK603 behaves as any other maize and that no unintended effects introduced by the event that might compromise the safety of this maize line were present.

**MON810:** Reports of rat and broiler feeding studies, generated after the original EU approval was granted for MON810 maize were provided. Overall, these additional data further support the original conclusion that led to market approval, that MON810 behaves as any other maize variety.

**NK603 X MON810:** Poultry feeding studies were conducted to demonstrate the safety of NK603 X MON810 maize grain as animal feed. The results confirm that hybrid maize NK603 X MON810 is nutritionally equivalent to its non-transgenic counterpart and therefore support earlier conclusions regarding the safety of NK603 and MON810 maize grain.

Overall, the animal feeding data assessed by ACAF support the view that the kernels from both of the parental maize lines (NK603 and MON810) behave as any other maize when used in the diets of animals. ACAF is satisfied that the safety of the parental maize lines and of the hybrid maize line has been established.

ACAF is content that the compositional equivalence of NK603 X MON810 grain and taken together with the results of the rat and poultry studies, these data suggest that grain from NK603 X MON810 would behave as any other equivalent variety of maize and would not pose a risk to livestock or consumers of livestock products.

#### **Environmental risk assessment**

ACRE considered carefully the environmental risk assessment for NK603 X MON810 provided by the notifier. The Committee noted that the scope of this application was for consent to import and use (but not to cultivate) the harvested F2 grain in the EU. The genetic modification involves two well-established genes that have a history of safe use.

The notifier presented evidence for the genetic stability of the inserts in hybrid maize. ACRE is satisfied with the Company's conclusion regarding the negligible likelihood and potential consequences of recombination between the two non-allelic inherited genetic sequences during mitosis and meiosis in NK603 X MON810 maize and that this applies to both F1 hybrid seed and plants as well as to the fraction of F2 grains containing both inserts following Mendelian segregation.

The environmental risk assessment does not identify any potential differences between conventionally bred NK603 X MON810 hybrid maize and non-transgenic maize varieties for phenotypic characteristics, with the exception of the two new characteristics, tolerance to glyphosate and insect resistance. ACRE considered the potential for gene dissemination and gene transfer from

NK603 X MON810. No differences in dissemination capacity or increased potential for gene transfer have been observed in pollen, seed and vegetative material from NK603 X MON810 compared with non-GM maize. Since maize does not establish properly outside the agricultural environment, the impact of escape of grain during storage or transport on gene transfer into other maize crops or weeds was considered to be extremely low. Members considered that because of the low germination rate and subsequent low viability of any germinated maize volunteer plants there were no anticipated environmental risk problems.

### **Post-market monitoring**

The aim of the case-specific part of the post-market monitoring plan (PMMP) is to investigate any risks identified in the environmental risk assessment, and to test any assumptions made in the risk assessment. ACRE agrees that on the basis of the risk assessment for NK603 X MON810 there is no requirement for case-specific monitoring. For the general surveillance part of the PMMP the notifier proposes to make use of those people and their networks that are responsible for transport, processing and handling of the GM maize grain. Monsanto proposes to submit reports of the outcome of this monitoring on an annual basis after authorisation. Although ACRE were content with the general surveillance aspects of the PMMP, the committee recommends that provision of the detailed arrangements for general surveillance should be made a condition of any consent. These further details should include: (1) precisely who will be requested to provide information; (2) what type of information will be requested and the frequency of requests and (3) how the Company will ensure participation to ensure a robust assessment.