



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 and processing of grain derived from hybrid maize containing events NK603 and MON810. This notification excludes cultivation and use as feed.
Date: **9 March 2006**

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 previously advised that sufficient information had been provided by Monsanto to demonstrate that the import and processing of NK603 X MON810 maize in the EU does not pose any greater risk to human health and the environment as compared to non-GM maize (see below: ACRE advice issued 30 January 2004). ACRE has now considered the Opinion of the EFSA GMO Panel on this notification and concludes that there is no new information that alters this advice and that the Panel's Opinion is in agreement with ACRE's advice.

The original scope of this notification (considered under Directive 2001/18/EC) included feed use. However, Monsanto has since submitted a separate application for NK603 X MON810 maize for food and feed use (under Regulation EC/1829/2003), thus the scope of the application is now reduced to import and processing of grain only.

The cultivation of NK603 X MON810 maize is not included in the scope of this notification and is therefore not addressed in this advice.

The Committee concludes that the Opinion of the EFSA GMO Panel is aligned with ACRE's view that it is unlikely that NK603 X MON810 maize will have any adverse effect on human and animal health or the environment in the context of its proposed uses.

Comment

The European Food Safety Authority (EFSA) has published its scientific opinion on the Part C marketing application for NK603 X MON 810 maize since our last advice was issued in January 2004 (see below).

This GM maize is a cross between two existing GM maize lines (NK603 and MON810) that already have marketing approval in the EU. NK603 is modified for tolerance to the herbicide glyphosate and MON810 is modified for insect resistance.

The UK is the lead Member State for this notification under Directive 2001/18/EC and issued a positive assessment in March 2004 (in line with ACRE's advice below).

The scope of this notification originally included feed use. The GMO subgroup of the Advisory Committee on Animal Feedingstuffs (ACAF) concluded that the import, processing and use of grain from NK603 X MON810 maize as animal feed in the EU does not pose any greater risk to human health and the environment as compared to its non-GM counterparts. Monsanto has since submitted a separate application for NK603 X MON810 maize for food and feed use (under Regulation EC/1829/2003), thus the scope under Directive 2001/18/EC has been reduced to import and processing of grain only.

We have now considered the Opinion from the EFSA GMO Panel on this notification and conclude that no new information has come to light that alters our previous advice.



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 has 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 *NdeI* 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

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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

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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.