



**ADVISORY COMMITTEE ON RELEASES TO THE ENVIRONMENT**

***Advice on a notification for marketing of herbicide tolerant GM maize***

- Notifier:** Monsanto Europe S.A.
- Notification Reference:** C/ES/00/01
- Product:** Maize genetically modified for tolerance to the herbicide glyphosate, line NK603.
- Scope:** For the import and use of grain varieties derived from maize event NK603, and conventional hybrids, as for any other maize excluding cultivation.
- Date:** 19<sup>th</sup> August 2003

**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).**

**Secondary advice:** ACRE is satisfied at this stage on the basis of the evidence provided that the risk to human health and the environment arising from marketing 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) and considered the notifiers responses to three requests for further information (see Appendix 1). The responses provided were adequate and ACRE sees no reason that consent to market NK603 maize should not be given.

**Comment**

ACRE considered the risks to human health and the environment posed by the release of genetically modified herbicide tolerant maize.

In its primary advice (Appendix 1) the committee requested three pieces of further information from the notifier:

1. The sequences of the DNA flanking the single insertion site of the expression cassette should be provided together with a sequence comparison analysis.  
The requested information was provided by the notifier in full. ACRE considered the flanking sequence data and the bioinformatic analysis provided by the notifier. The Committee was content that these data and analysis confirmed that the inserted DNA had not disrupted endogenous genes, and there were no endogenous regulatory sequences nearby. As a result ACRE concluded that they were now content with this aspect of the risk assessment.
  
2. Full data on both rat and the broiler chick experiments.  
In the original notification the notifier had stated the conclusions of two animal feeding studies, but had not provided the data. The notifier has now provided the data as requested, and both ACRE and ACAF are content that the data supports the notifiers conclusions – that NK603 maize behaves as any other maize in respect of animal feed safety.
  
3. Completion of the statistical evaluation of the remaining two European trial sites and a meta-analysis across all four EU sites.  
The Committee requested completion of the statistical analysis. This was provided and did not indicate any significant compositional differences between NK603 and other maize.  
The committee also requested a full meta-analysis of compositional studies across all 4 EU trial sites. The notifier argued that it was not possible to do a meta-analysis due to the design of some of the trials. ACRE and ACAF agree that a conventional analysis of variance is not possible because of this limitation, but it is of the opinion that there are other approaches of meta-analysis that are suitable. ACRE does not consider this an essential requirement, but recommends that such a statistical analysis is carried out.



## APPENDIX 1

Primary ACRE Advice, 7<sup>th</sup> March 2003

### ***ADVISORY COMMITTEE ON RELEASES TO THE ENVIRONMENT***

#### ***Advice on a notification for marketing of herbicide tolerant GM maize***

- Notifier:** Monsanto Europe S.A.
- Notification Reference:** C/ES/00/01
- Product:** Maize genetically modified for tolerance to the herbicide glyphosate, line NK603.
- Scope:** For the import and use of grain varieties derived from maize event NK603, and conventional hybrids, as for any other maize excluding cultivation.
- Date:** 7<sup>th</sup> March 2003

**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).**

**Primary advice:** ACRE is satisfied at this stage on the basis of the evidence provided that the risk to human health and the environment arising from marketing 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. This advice is pending receipt of further information as outlined below. In coming to this conclusion ACRE have taken account of the advice of the Advisory Committee on Animal Feedstuffs (ACAF) who have recommended that further data be sought on the animal studies performed in the assessment of this line. ACRE also note that information on the flanking sequences of the insertion site were not provided and while taking this into account during its assessment advise that this information should be requested. Further information required is as follows:

1. The sequences of the DNA flanking the single insertion site of the expression cassette should be provided together with a sequence comparison analysis.
2. Full data on both rat and the broiler chick experiments.
3. Completion of the statistical evaluation of the remaining two European trial sites and a meta-analysis across all four EU sites.

## Comment

This notification was received by the UK from the Spanish Authorities (via the Commission) following their risk assessment of this GM maize. ACRE considered the notification and the potential risks arising from importation and commercial use of this GM maize, excluding cultivation. In arriving at its advice the Committee considered the notification against the requirements of the legislation as it relates to the UK and in particular:

- Capacity to survive, establish and disseminate.
- Potential for gene transfer
- Genetic and phenotypic stability
- Expressed products from the inserted sequences
- Potential adverse effects for humans and animals: toxic and allergenic effects.
- Interactions with other organisms.
- Potential effects on biogeochemical processes
- Impacts resulting from changes in management practices associated with herbicide tolerant crops.

In respect of each of these assessment criteria ACRE concurs with the conclusions of the Spanish Authorities and the underpinning reasoning, but has the following additional comments:

### Effects on biogeochemical processes and impacts arising from changes in management practices

Potential effects on biogeochemical processes, or impacts resulting from changes in management methods, arising from cultivation of the herbicide tolerant maize do not apply directly to this notification in which the GM maize is not for cultivation. The likelihood of any potential effects being manifest on biogeochemical processes arising from unintentional introduction of the GM maize into the environment was considered to be extremely low.

### Molecular characterisation

ACRE considered the molecular information provided to be of good quality and agreed with the assessment that a single copy of the DNA fragment used in the transformation, containing two *cp4 epsps* gene cassettes had been inserted into the genome of maize NK603. The transformation had been performed using a fragment of plasmid PV-ZMGT32L and ACRE was content on the basis of the evidence provided that the genome of NK603 maize does not contain detectable plasmid backbone sequences.

ACRE noted that an extra 217 bp fragment of the enhancer region of the rice actin promoter (*P-ract1*) was also inserted at the 3' end of the transformation cassette. The notifier states that sequences required for the promoter to function and initiate transcription (TATA box and transcription initiation site) were not included in the inserted 217 bp fragment and no transcription product was detected that initiates in genomic DNA flanking the 3' end of the inserted DNA.

In contrast, a transcription product was detected which initiated from the NK603 insert and extends beyond the NOS 3' genetic element. The notifier states that this

transcription product terminates in the genomic DNA flanking the 3' end of the inserted DNA.

ACRE was content that neither of these features altered the risk assessment of the maize NK603.

Some information is provided in the dossier on the insertion site, with PCR data of the insertion site sequences indicating that a three base pair deletion had occurred upon insertion of the DNA and allowing the conclusion that the DNA sequences flanking the 5' and 3' ends of the insert were native to the maize genome. ACRE request further information in this regard, specifically the sequence of the DNA flanking the insertion site. A sequence comparison analysis of the flanking DNA for similarity for known genes, expression products and functions is also desirable.

#### Animal feed safety

ACRE asked the Advisory Committee on Animal Feedings (ACAF) to assess NK603 from the perspective of animal feed safety. ACAF considered the dossier with respect to the safety of the expressed proteins, safety of kernels from maize line NK603 and animal studies. ACAF's advice to ACRE is summarised here and is appended to this advice.

#### *Safety of the expressed proteins*

ACAF noted that the EPSPS protein produced in bulk in *E. coli* has been used in both acute and chronic repeat dose toxicity studies (the latter not reported in this summary dossier). These studies are not specific to this particular event but are, and have been, used for other products transformed to express the form of *epsps* derived from *Agrobacterium* strain CP4. Only the comparisons of sequence homology could be said to be event specific since they were implied to include the L214P variant.

Taking into account the growing safe history of use of EPSPS ACAF advise that although it would not be possible to come to a conclusion on the safety of the introduced and expressed proteins for livestock or for humans simply on the basis of the information presented in this dossier alone, there is a substantial body of data on CP4 EPSPS establishing its inherent safety. This evidence has been considered sufficient in the past. There is no reason to suppose that the two CP4 EPSPS proteins produced by the maize line NK603 behave any differently to CP4 EPSPS produced in other maize lines or in other commodity crops.

#### *Safety from kernels from maize line NK603*

In considering the quality of the comparative compositional data ACAF noted that the design of the experiment may have lead to some of the variation seen. ACAF concluded that other than a small increase in total fat in the GM variety grown in Europe compared to the control and a higher moisture content in the control line when data was pooled across the US trials, proximate analysis showed no significant differences between the test crop and its control. The variation in results from the more detailed analysis of constituents was that expected of studies made across growing seasons and geographical areas. No consistent differences that would suggest an unintended effect on the plants' metabolism were evident. Consequently, the composition of the kernels from maize line NK603 can be considered as

substantially equivalent to its parental line and would be expected to behave as any other maize line when fed to animals.

### *Animal studies*

Rat feeding studies indicated no differences in weight gain or feed consumption were reported for the GM and its non-GM counterpart. Haematological and clinical chemistry parameters were “generally comparable between NK603 and the parental control. The few differences that were observed were described as “generally of small magnitude, inconsistently observed and not dose-related”. There was similarly said to be no evidence of treatment-related macroscopic or microscopic differences between animals fed the control and test diets. Whilst this is stated by the notifiers, no data to support these conclusions was presented with the description of this study and it is not possible to confirm the accuracy of the conclusions drawn by the Company.

The same lack of data applies also to the second study using broiler chicks which also purported to show that, in this case, chicks fed the GM line thrive and grow at the same rate as those fed the non-GM parental line and that this is further evidence of the equivalence of NK603 to other maize lines.

ACAF consider that while the data taken at face value would suggest that NK603 behaves as any other maize and would present no cause for concern for either animals fed the grain or for consumers of products derived from animals fed NK603, the data provided on these experiments is inadequate and it is not possible to confirm the interpretation of the company.

It would also be helpful if the Company could complete its statistical evaluation of the remaining two European trial sites and to make a meta-analysis across all four EU sites.

### Overall Conclusions

ACRE advise at this stage that marketing of this product for importation purposes poses very low risk to human health and the environment but that the Committee would like to see sequence data for the insertion site flanking sequences and information on the animal feeding studies. ACRE advised that the Committee had noted the absence of this information during its safety assessment and had taken this into account, but also considered that this data would be useful and would underpin further the risk assessment process.