



ADVISORY COMMITTEE ON RELEASES TO THE ENVIRONMENT

Advice on a notification for marketing of insect resistant maize

Notifier: Monsanto Europe S.A.
Notification reference: C/DE/02/9
Product: Maize genetically modified for insect resistance, lines MON 863 and MON 863 x MON 810.
Scope: For the import and use of grain varieties derived from event MON 863 and MON 863 x MON 810 hybrids, as for any other maize excluding cultivation.
Date: 14 January 2008

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).

Further Advice on MON 863: ACRE has previously considered this notification. Marketing consent for MON 863 was issued February 2006. Since this time, EFSA has published two statements relevant to the insect resistant event MON 863 maize.

(i) *EFSA statement on the use of *nptII* as a selective marker gene.* ACRE has considered recent documents from the EMEA and from EFSA on the safety of the *nptII* gene that is present in MON 863 maize. ACRE considers that these documents do not present any new information. Consequently, ACRE retains its view that the import and use of MON 863 maize poses no additional risks to human health or the environment as compared with its non-GM parental cultivar. In coming to this conclusion ACRE has taken account of the advice of the Advisory Committee on Animal Feedingstuffs (ACAF).

(ii) *EFSA GMO Panel review of the statistics applied to a rat feeding study with MON 863 maize.* ACRE has assessed the re-evaluated MON 863 rat feeding study data and concludes that these data do not present new information that would require the Committee to change its previous advice on import and use of MON 863. In coming to this conclusion ACRE has taken account of the advice of the Advisory Committee on Animal Feedingstuffs (ACAF).

Overall, ACRE advises that the safety of MON 863 remains unchanged and that the import and use of MON 863 maize poses no additional risks to human health or the environment as compared with its non-GM parental cultivar.

(i) EFSA statement on the use of *nptII* as a selective marker gene

Background

Since ACRE last considered this notification, the European Medicines Agency (EMA) has issued a report¹, which concludes that neomycin and kanamycin are of importance for veterinary and human use. Neomycin and kanamycin are antibiotics that are substrates of the NPTII protein, which is produced in MON 863 GM maize as a result of genetic modification. The EMA report considers the potential for horizontal gene transfer from GM plants to bacteria² and the prevalence of *nptII* genes already present in the environment.

EFSA has provided a statement³ in response to the report issued by the EMA. In this statement, EFSA agrees that the preservation of the therapeutic potential of the aminoglycoside group of antibiotics is important. However, it concludes that the therapeutic effect of antibiotics that are substrates for NPTII will not be compromised by the presence of the *nptII* gene in GM plants.

Comment

In previous assessments of MON 863 maize ACRE considered the environmental implications associated with the presence of the *nptII* gene in this GM maize (please refer to previous ACRE advice issued in 2003). The EMA and EFSA documents do not introduce any new information or raise any concerns that we were not aware of when we issued this advice. ACRE is in full agreement with EFSA's conclusion that the therapeutic effect of antibiotics that are substrates for NPTII will not be compromised by the presence of the *nptII* gene in GM plants. ACRE agrees with EFSA that (a) the likelihood of transfer of a functional gene from plant material to bacteria is extremely low; (b) bacteria with resistance to these antibiotics are widespread in the environment; and (c) acquisition of an intact gene is only one of the possible mechanisms for bacteria to develop resistance.

(ii) EFSA GMO Panel review of the statistics applied to a rat feeding study with MON 863 maize

Background

In March 2007, a group of concerned scientists published a statistical analysis of the results of the 90-day rat feeding study that had formed part of the application dossier for MON 863 maize⁴. Seralini *et al.* concluded that rats fed MON 863 maize kernels showed a number of statistically significant differences, including some that were

¹ EMA (2007) Presence of the antibiotic resistance marker gene *nptII* in GM plants for food and feed uses (<http://www.emea.europa.eu/pdfs/human/opiniongen/5693707en.pdf>)

² The EMA considers that their area of competence does not extend to a detailed consideration of the likelihood of transfer of antibiotic resistance genes from plant material to bacteria of man and animals.

³ Statement of the Scientific Panel on Genetically Modified Organisms on the safe use of the *nptII* antibiotic resistance marker gene in genetically modified plants (http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620775641.htm)

⁴ Seralini, G.E., Cellier, D., de Vendomois, J., S., 2007. "New analysis of a rat feeding study with a genetically modified maize reveals signs of hepatorenal toxicity". *Arch. Environ. Contam. Toxicol.*, 52, 596-602. Available from: <http://springerlink.metapress.com/content/100119/>

indicative of liver and/or kidney toxicity. The European Commission sought advice from EFSA on this new analysis and EFSA's response was published on 25 June 2007, available at: <http://www.efsa.europa.eu/en/science/gmo/statements0.html>. EFSA's GMO Panel considered the biological significance of all statistically significant differences in the test parameters and has advised that this publication does not raise any new issues that are toxicologically relevant. EFSA therefore confirmed its previous opinion that "MON 863 maize would not have an adverse effect on human or animal health ... in the context of its proposed use". EFSA also published a detailed statistical assessment of the data from the rat feeding study, which is available via the Scientific Reports area of the EFSA website: http://www.efsa.europa.eu/en/science/scientific_reports/statistical_analyses_MON863.html. The latest statement from EFSA can be found at: http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178621165358.htm.

Comment

Both ACRE and ACAF committees were consulted independently for their advice on the EFSA statement.

The GM sub-group of ACAF was consulted in relation to the safety of MON 863 as it is an approved product for use as animal feed. It has provided scientific advice over several years on this product and has always concluded that it has no concerns for the safety of MON 863 for animal feed purposes.

The view of ACAF is that the EFSA review of the statistics applied to the MON 863 rat study is thorough and competent. It agrees with the EFSA conclusion that the statistically significant differences seen in body weights at various times during the 90 day study were probably attributable to fluctuations in food intake and not indicative of an adverse response. Similarly, ACAF agree that differences seen in the various (bio)chemical parameters and organ weights measured and highlighted in the paper by Séralini *et al.* arose largely by chance and, as it has previously concluded, these are without toxicological significance.

Consequently, ACAF sees no reason to revise its original opinion on the safety of MON 863 when used for animal feed purposes.

ACRE considered the same data and information that had been provided to ACAF, with a specific remit to consider environmental risks and not food and feed safety. ACRE's independent assessment of the information concluded that the re-evaluated rat feeding data did not present new information that would require the Committee to change its previous advice on import and processing of MON 863 maize and that its opinion was in line with the conclusions of EFSA and ACAF.



ADVISORY COMMITTEE ON RELEASES TO THE ENVIRONMENT

Advice on a notification for marketing of insect resistant maize

Notifier:	Monsanto Europe S.A.
Notification reference:	C/DE/02/9
Product:	Maize genetically modified for insect resistance, lines MON 863 and MON 863 x MON 810.
Scope:	For the import and use of grain varieties derived from event MON 863 and MON 863 x MON 810 hybrids, as for any other maize excluding cultivation.
Date:	7 September 2005

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).

Revised advice after the 45-day assessment period: ACRE has previously considered this notification (advice issued 12 June 2003 and 13 October 2003, see Annex 1). Since issuing this advice, Monsanto Europe S.A. has submitted a new rat feeding study performed with insect resistant hybrid maize MON 863X MON 810. ACRE has considered this further information and conclude that the new study gives no reason to change their previous opinion, i.e., that this GM maize does not pose a risk to human health and the environment and that 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).

Comment

ACRE re-considered the risks to human health and the environment posed by the marketing of genetically modified insect resistant maize in the light of new data provided by the notifier on the hybrid insect resistant maize. ACRE asked the Advisory Committee on Animal Feedingstuffs (ACAF) to assess the new MON 863 x MON 810 hybrid study from the perspective of animal feed safety. ACAF previously considered the animal feed safety assessments of both MON 810 and MON 863 and they were found to be as safe as any other maize lines when fed to animals, and thus the safety of the hybrid (MON 810 X MON 863) could be presumed. This view was supported and strengthened by the results of the second broiler growth study in which birds were fed the hybrid maize line.

As rat feeding studies had been provided with respect to the two individual lines (MON 863 and MON 810) and were considered to support the safety of these two lines, ACAF did not consider necessary a third feeding study made with the hybrid. However, such a study was requested by EFSA. The full study report has been assessed by ACAF. The Committee considers the study to be of good design and well- conducted and reported. The limited differences which were identified between control and MON 863 X 810 hybrid treated rats in the new study are likely to have arisen by chance and thus give no reason to alter the opinion previously expressed regarding safety of grain from this hybrid.



ADVISORY COMMITTEE ON RELEASES TO THE ENVIRONMENT

Advice on a notification for marketing of insect resistant maize

Notifier: Monsanto Europe S.A.
Notification reference: C/DE/02/9
Product: Maize genetically modified for insect resistance, lines MON 863 and MON 863 x MON 810.
Scope: For the import and use of grain varieties derived from event MON 863 and MON 863 x MON 810 hybrids, as for any other maize excluding cultivation.
Date: 12th June 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 has considered the information provided in this dossier and the Committee is satisfied, at this stage, that there is no evidence to suggest that the marketing of this product for importation and processing in the UK poses a risk to human health and the environment. However in order to make a full assessment of this GM product and before an opinion as to whether this product should be marketed in the EU is made ACRE requests further information as detailed below. In coming to this conclusion ACRE has taken account of the advice of the Advisory Committee on Animal Feedingstuffs (ACAF).

1. A consideration of the molecular characterisation of parental line MON 810, and in particular an assessment of risks associated with the possible presence of multiple inserts and vector backbone sequence.
2. Full data concerning the broiler-chick feeding study with MON 863 maize kernels.
3. A risk assessment for the MON 863 x MON 810 hybrid taking into account the fact that it will be the F2 generation which will be imported and that there may be a very small opportunity for recombination. Any uncertainties should consequently be taken into account in the post-market monitoring plan.

Comment

This notification was received by the UK from the German 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 within the context of the scope of the notification.

Molecular characterisation

ACRE considered the molecular information relating to MON 863 provided in the dossier to be well structured and generally of a high quality. This dossier provides a good example of how molecular characterisation of a GMO for marketing purposes should be presented. While the quality of the reproduction of the southern blots was just adequate for a considered assessment to be made the Committee stresses the importance of providing high quality copies or electronic versions of such blots.

ACRE agree with the assessment by Monsanto that MON 863 contains a single copy of the *MluI* DNA fragment used in the transformation, containing the *cry3Bb1* and *nptII* cassettes inserted into the maize genome. ACRE noted the presence of a truncated *ble* gene downstream of the *nptII* and is content that no significant expression of this partial gene will occur in the genetically modified maize. The transformation had been performed using a fragment of plasmid PV-ZMIR13 and ACRE was content that the evidence provided supports the conclusion that the genome of MON 863 maize does not contain detectable plasmid backbone sequences.

Monsanto have provided information regarding the genomic DNA sequences flanking the 5' and 3' ends of the insert in corn rootworm event MON 863. PCR and DNA sequence analysis have identified 242 and 224 bp that flank the 5' and 3' ends respectively of the insert. Disappointingly Monsanto did not provide details that these sequences were native to the maize genome or a sequence comparison analysis for similarity to known genes, expression products and functions. However the German competent authority have carried out a comparison of the sequences of the flanking regions with public sequence databases and concluded that the 5' region yields a 99% homology with exon 4 of the mitochondrially coded *Zea mays* NADH dehydrogenase subunit. ACRE is content with this conclusion.

Information directly relating to the molecular characterisation of MON 810 is not presented in this dossier and the reader is referred to consent C/F/95/12-02 issued in April 1998 by France for the cultivation and use of MON 810 as for any other maize. Recently, ACRE has reconsidered data provided concerning MON 810 as part of its assessment of the hybrid NK 603 x MON 810 – the UK is lead

competent authority on an application for import of this hybrid (reference: C/GB/02/M3/3). On the basis of the information provided in support of application C/GB/02/M3/3, ACRE considers that further consideration of the risk assessment of MON 810 is appropriate. In particular, the Committee does not accept that the evidence provided unambiguously establishes that the vector backbone is absent and that the copy number of the insert is one. While ACRE does not consider that this necessarily implies additional risk, the Committee nonetheless requests that the applicant take this into account in their risk assessment of MON 810.

Presence of the *nptII* gene

Transformation event MON 863 contains a functional *nptII* gene conferring resistance to the antibiotics neomycin and kanamycin. ACRE recognises that antibiotic resistance marker genes raise concerns because of the impact that they may have on antibiotic resistance in bacterial populations, and has advised that antibiotic resistance marker genes should be avoided in the design of GM crops¹. However, the Committee is also of the view that the presence of specific antibiotic resistance markers in particular genetic contexts should be considered on a case-by-case basis. In this regard, ACRE has advised on the use of the *nptII* gene previously, concluding that this marker gene does not constitute a risk to human health or the environment. The Committee endorses this view with reference to the presence of the *nptII* gene in line MON 863. The reasons for this conclusion are:

1. The available evidence suggests that rates of horizontal gene transfer between plants and bacteria are low.
2. The *nptII* gene in MON 863 is driven by a plant virus promoter, rather than a bacterial promoter so that expression rates would be low should the gene transfer to bacteria
3. The *nptII* gene is already widespread in natural bacterial populations, so the contribution that the release of MON 863 would make to overall resistance levels in bacteria in the environment is negligible.
4. Kanamycin and neomycin are of limited therapeutic value, because of the current prevalence of resistant bacteria and the mammalian toxicity of these antibiotics. It is noted that neomycin is used as a topical agent in various ointments for skin infections and in eye drops.

Animal feed safety

ACRE asked the Advisory Committee on Animal Feedingstuffs (ACAF) to assess MON 863 and MON 863 x MON 810 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 lines MON 863 and MON 863 x MON 810 and animal studies. ACAF's advice to ACRE is detailed here.

• ¹ Guidance on Principles of Best Practice in the Design of Genetically Modified Plants. Available at <http://www.defra.gov.uk/environment/acre/bestprac/index.htm>

Safety of the expressed proteins

The Cry3Bb1 and NPTII protein used for safety studies was obtained from overexpression in *E. coli*. Evidence of a close similarity between the proteins derived from a bacterium and the corresponding products expressed in maize is provided. *In vitro* studies of Cry3Bb1 derived from *E. coli* and from MON 863 using a simulation of digestion by gastric fluid showed that both sources of the protein were degraded within 15 seconds and that the small peptides produced could no longer be detected after 15 minutes. These results suggest that Cry3Bb1 would be rapidly degraded in the mammalian digestive tract. An acute oral toxicity study in mice administered Cry3Bb1 protein did not show any adverse effects in terms of mortality, clinical observations, body weight gain or food consumption. Necropsy after 14 days also failed to indicate adverse effects attributable to the test material. Similar acute studies have been previously reported for NPTII, however, exposure to NPTII from MON 863 is very low. Equivalent studies have also been made with Cry1Ab in pursuit of approval for MON 810.

Safety of kernels from maize lines MON 863 and MON 863 x MON 810

Comparative compositional data is described using MON 863 grain collected from the USA in 1999 and Argentina in 1999/2000 and for the insect-resistant hybrid collected from Argentina in 1999/2000. In all cases the GM line is compared with the unmodified hybrid (MON 846), to a number of commercial non-transgenic hybrids and with the literature. The data presented shows that both MON 863 and the MON 863 x MON 810 hybrid are very similar to their corresponding controls and have a composition typical of existing commercial hybrids. On the basis of these data they would be expected to deliver essentially the same nutrition when fed to livestock.

Data on the composition of MON 810 was presented in the previous application made for this line by the Company under Directive 90/220/EEC in 1995.

Animal studies

The summarised results of a feeding study made with diets including MON 863 maize kernels in comparison with diets containing comparable amounts of a near isogenic control and six non-transgenic commercial hybrids fed to broiler chicks for a period of 42 days is briefly described. No significant differences in performance parameters were noted apparently indicating that the transgenic line delivered the nutrition indicated by its composition and that no unrecognised adverse changes had been introduced. However, insufficient data on the numbers of birds used, the experimental design (number of experimental units) and the diet composition were provided to confirm the conclusion recorded by the Company.

The results of a 13-week feeding study made with MON 810 kernels fed to rats is also presented. In summary body weight gain and food consumption was comparable with rats fed MON 810 and those fed the unmodified maize line and no differences attributable to the test article were found amongst either the

clinical pathological parameters measured or on examination of tissues. Comparable data for MON 863 was not made available.

Characterisation of the hybrid

ACRE recognises that in order to assess the MON 810 x MON 863 hybrid Monsanto have carried out an assessment of the two parental lines separately and provided additional information eluding to the absence of interactions between the newly introduced genetic material. The only data provided in the dossier regarding potential interactions of the newly introduced genetic material is in the compositional data analysis, on the basis of which the general safety of the hybrid is supported.

However ACRE requests that the risk assessment should be revised to take into account the fact that it will be the F2 generation which will be imported, not the F1, and that there may be a very small opportunity for recombination. While it is ACRE's assessment that the risks arising from potential recombination are low, an assessment of this should be included. The revised environmental risk assessment should then be used in a reconsideration of the post-market monitoring plan, which should be devised to underpin assumptions made in the risk assessment.

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 is considered to be extremely low.



ADVISORY COMMITTEE ON RELEASES TO THE ENVIRONMENT

Advice on a notification for marketing of insect resistant maize

Notifier: Monsanto Europe S.A.
Notification reference: C/DE/02/9
Product: Maize genetically modified for insect resistance, lines MON 863 and MON 863 x MON 810.
Scope: For the import and use of grain varieties derived from event MON 863 and MON 863 x MON 810 hybrids, as for any other maize excluding cultivation.
Date: 13 October 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 has considered this notification for the import and use of insect resistant maize (event MON 863 and hybrids of MON 863 x MON 810). The committee considers that sufficient information has now been provided by the notifier to demonstrate that this GM maize does not pose a risk to human health and the environment and that 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 Feedings (ACAF).

ACRE considered the notifiers responses to three requests for further information (see Appendix 1). ACRE was satisfied with the responses provided regarding molecular characterisation of MON 810, animal feeding studies and the risk assessment for the F2 generation of MON 863 x MON 810.

However the committee is still not satisfied with the post market monitoring plan and requires that the notifiers be proactive in obtaining information from end users of MON 863 and MON 863 x MON 810 that are attributable to the use of the GMO which should then form part of an annual report to the authorities.

Comment

ACRE considered the risks to human health and the environment posed by the marketing of genetically modified insect resistant maize. In its primary advice (Appendix 1) the committee requested three pieces of further information from the notifier:

1. A consideration of the molecular characterisation of parental line MON 810, and in particular an assessment of risks associated with the possible presence of multiple inserts and vector backbone sequence. ACRE considered the additional information provided by the notifier and agree that the data support the conclusion that vector backbone sequences are absent and that the copy number of the insert is one.
2. Full data concerning the broiler-chick feeding study with MON 863 maize kernels. The requested information was provided by the notifier in full. The animal feeding studies provided support the view that MON 863 delivers the nutrition expected on the basis of its chemical composition and no adverse unintentional changes have been introduced by the transformation event. ACAF concludes that the safety assessment of both MON 863 and MON 863 x MON 810 maize lines has been completed and both have been found to be safe as any other maize line.
3. A risk assessment for the MON 863 x MON 810 hybrid taking into account the fact that it will be the F2 generation which will be imported and that there may be a very small opportunity for recombination. Any uncertainties should consequently be taken into account in the post-market monitoring plan. ACRE considered the notifiers revised risk assessment taking into account the potential for recombination between the two parental lines MON 863 and MON 810 in the F2 generation. The two transgenes share limited sequence homology and each appears to be inherited in a stable manner indicating that there is unlikely to be any significant frequency of recombination of the individual genes in the parental lines. Hence recombination of the two inserts in the F2 generation would be very rare and no increased hazard is expected.

Post Market Monitoring plan

ACRE considered the revised post market monitoring plan provided and considers that the applicant should be more proactive in obtaining information from end users of any adverse effects that are attributable to the use of the GMO which should be combined with an annual reporting requirement