



ADVISORY COMMITTEE ON RELEASES TO THE ENVIRONMENT

Advice on a notification for marketing of a GM potato modified for starch composition

Notifier:	BASF Plant Science
Notification reference:	C/SE/96/3501
Product:	Potato clone EH92-527-1 genetically modified to enhance the content of the amylopectin component of starch.
Scope:	Cultivation of potato clone EH92-527-1 and use for the extraction of starch for industrial uses.
Date:	20 May 2007

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: ACRE has considered recent documents from the EMEA and from EFSA on the safety of the *nptII* gene that is present in potato clone EH92-527-1. ACRE considers that these documents do not present any new information. Consequently, we retain our view that the cultivation and use of potato clone EH92-527-1 poses no additional risks to human health or the environment as compared with its non-GM parental cultivar.

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 GM potato clone EH92-527-1 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.

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 To note that 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.

EFSA has provided a statement³ in response to the report issued by the EMEA. 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 our original assessment of notification C/SE/96/3501, ACRE considered the environmental implications associated with the presence of the *nptII* gene in this GM potato (please refer to our advice in Appendix A). The EMEA 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.

³Statement 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/etc/medialib/efsa/science/gmo/statements/npt2.Par.0001.File.dat/gmo_state ment_%20nptII_.pdf)

Annex 1

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Notifier:	BASF Plant Science
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Product:	Potato clone EH92-527-1 genetically modified to enhance the content of the amylopectin component of starch.
Scope:	Cultivation of potato clone EH92-527-1 and use for the extraction of starch for industrial uses.
Date:	18 April 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).

Further advice: ACRE has considered the further information provided to the EFSA GMO panel concerning this notification for marketing of potato clone EH92-527-1, modified for the production of high amylopectin starch. On the basis of the information provided and the modified scope of this application, the Committee holds the view that cultivation and use of potato clone EH92-527-1 poses no additional risks to human health or the environment compared to the parental potato cultivar.

Comment

Scope

The scope of the application has been reduced since the last assessment to exclude the use of the by-products of starch extraction in animal feed.

Further information on the stability of the insert, bioinformatics analysis and molecular characterisation

In its previous consideration of this application (advice attached at Annex 2), ACRE concluded that no significant risk to human health or the environment had been indicated by the molecular characterisation of this potato clone. The applicant provided further information on the presence of open reading frames and the stability

of the insert in response to a request from the EFSA GMO panel. ACRE concluded that the results of the Southern Blot analysis demonstrated that the insert was stable over several generations. BLAST searches revealed that two open reading frames (ORF12 and ORF14) have significant homologies to new database entries. The Committee was content that these homologies were the result of *nptII* sequence and plasmid sequence on the T-DNA used for transformation of this potato clone. The Committee concluded that the new information concerning the stability of the insert and bioinformatics analysis were consistent with the conclusions of the applicant and did not indicate any risks to human health or the environment.

Impact on Non-target organisms

The EFSA GMO panel asked the applicant to provide information on the impact of cultivation of this GMO on plant-associated organisms from records of field studies carried out in member states. The notifier concluded that no consistent long-term effects caused by the GMO were identified on any of the taxa monitored. ACRE welcomed the provision of this information and accepted the conclusions of the applicant.

Agronomic Considerations

The Committee recommended that potato groundkeepers should be strictly controlled in the years after the cultivation of this GM potato to avoid presence in subsequent non-GM potato crops. The Committee note that this recommendation relates to labelling requirements for GM products and is not relevant to the assessment of this application with respect to human health or the environment.

Post Market Monitoring

After its first assessment of this application, the Committee recommended that the applicant should examine the male fertility of the potato clone EH92-527-1 through case-specific monitoring. The reason for this request was that BLAST searches revealed that ORF4 may produce a protein with a some sequence homology for a mite allergen. ACRE were content that there was no evidence that a protein resulting from ORF4 was translated in any potato tissue tested but that the applicant had not provided evidence that this protein was not expressed in pollen. In its second consideration of the application the Committee accepted the argument of the applicant that this potato clone has limited flower development and that pollen formation is extremely rare. The Committee therefore revised its former recommendation that case-specific monitoring of ORF4 polypeptide in pollen should be done.

The EFSA GMO panel requested the notifier to provide a separate farmer questionnaire to allow farmers to record any unusual observations, the applicant responded to this request and indicated that all farmers would be provided with a Field-plot Card-index on which various parameters of crop growth including flower development should be recorded.

The Committee welcomed the proposal of the applicant to monitor flowering in the post-market monitoring of potato cultivation. The Committee accepted that this monitoring was appropriate and expect that any reporting of unusual events will trigger case-specific monitoring in the usual manner.

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Product:	Potato clone EH92-527-1 genetically modified to enhance the content of the amylopectin component of starch.
Scope:	Cultivation of potato clone EH92-527-1 and use for the extraction of starch for industrial uses, and the use of the by-products of starch extraction in animal feed.
Date:	14 January 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).

Secondary advice: ACRE has considered the further information provided concerning this notification for marketing of potato clone EH92-527-1, modified for the production of high amylopectin starch. On the basis of the information provided, the Committee continues to hold the view that cultivation and use of potato clone EH92-527-1 poses no additional risks to human health and environment compared to the parental potato cultivar. However, in order to complete the risk assessment further information is required concerning the effect of feeding the pulp of potato clone EH92-527-1 to rats in a 90 day toxicological study.

Comment

Molecular characterisation

In its previous advice (Appendix A) ACRE requested that the notifier provided further information concerning the presence of potential novel open reading frames or disruption of existing open reading frames in the potato genomic sequence adjacent to the insert site. The requested information has been provided. The Committee notes that a novel chimaeric open reading frame of 69 amino acids has been generated. This open reading frame (ORF) has no similarities to known proteins or allergens and is unlikely to be expressed, as no known regulatory sequences are

present in the vicinity of the ORF. As a result the Committee is content that the presence of this novel ORF does not represent a significant risk to health and the environment.

Animal feed safety

In its previous consideration of this application the GM subgroup of the Advisory Committee on Animal Feedstuffs (ACAF) concluded that, on the basis of the data provided, there was no reason to consider that feeding the by-products of starch extraction from potato clone EH92-527-1 to animals poses any additional risks to animal health compared to the parental cultivar (see Appendix A). However, the ACAF GM subgroup considered that a comparative feeding study made with rats using potato pulp derived from EH92-527-1 and from the parental cultivar was appropriate. Completion of a study in which pulp is included to the maximum level permitted by nutritional requirements and at an intermediate level would provide a basis for concluding on the absence of any unintended effects. The subgroup notes the argument presented by the notifier but remains of the view that a comparative feeding study with rats is required. ACRE agrees with the assessment of the ACAF GM subgroup.

Post market monitoring

In its previous advice (Appendix A), the Committee recommended that monitoring for the presence of the ORF4 protein in pollen should be carried out. The Committee notes that the notifier argues that this is not possible given the low male fertility of potato clone EH92-527-1. The Committee accepts this argument, and therefore recommends that case-specific monitoring should examine the male fertility of potato clone EH92-527-1. If significant, unexpected pollen production is detected then the presence of ORF4 protein should be examined,

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Scope:	Cultivation of potato clone EH92-527-1 and use for the extraction of starch for industrial uses, and the use of the by-products of starch extraction in animal feed.
Date:	28 May 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).

Primary advice: ACRE has considered this notification for marketing of potato clone EH92-527-1, modified for the production of high amylopectin starch. On the basis of the information provided, the Committee concludes that cultivation and use of potato clone EH92-527-1 poses no additional risks to human health and environment compared to the parental potato cultivar. However, in order to complete the risk assessment further information is required concerning:

1. The presence of potential novel open reading frames or disruption of existing open reading frames in the potato genomic sequence adjacent to the insert site.
2. The effect of feeding the pulp of potato clone EH92-527-1 to rats in a 90 day toxicological study.

Comment

Molecular characterisation

ACRE considered that the molecular characterisation of potato clone EH92-527-1 presented by the notifier was generally of a high standard, well presented and fit for purpose. The Committee concluded that sufficient evidence had been provided to confirm that the insert is present as a single copy, and that only sequences contained

within the T-DNA borders of the transformation vector are inserted within the potato genome.

The Committee noted that a detailed analysis of potential novel open reading frames (ORFs) contained within the inserted sequences has been carried out, in particular focussing on the ORF termed ORF4 by the notifier. This sequence contains homology to the bleomycin (*ble*) resistance protein of Tn5 (50 amino acids of the 126 found in the full length *ble* gene) together with a section of the ornithine cyclodeaminase gene of *Agrobacterium*. The Committee concluded that sufficient evidence has been provided to show that, while this novel ORF is expressed at the RNA level, novel protein is not produced in potato leaves or tubers. Further, the Committee concluded, on the basis of the notifier's studies on expression in *E. coli*, that the ORF4 gene does not encode a functional bleomycin resistance protein.

The Committee considered the notifier's analysis of homology of the ORF4 sequence with known allergens. This analysis had been carried out in the year 2000, and a repeat of these homology searches revealed additional homologies not reported in the notification. In particular, there is a 4 amino acid homology within the ornithine cyclodeaminase gene to a mite allergen. However, the Committee concluded that the presence of this homology did not pose a risk for the following reasons. First, this is a very short stretch of homology, which is unlikely to result in an allergenic protein. Secondly, this stretch of homology is found in an extant gene from *Agrobacterium*, which is already widespread in the environment. Thirdly, there is no evidence that protein with an amino acid sequence derived from ORF4 is present in potato clone EH92-527-1, at least in the tissues where this has been examined. The Committee notes that an investigation of the presence of ORF4 protein in pollen has not been carried out, but concludes that it is unlikely that the protein would be produced, and even if the protein were expressed in pollen this does not represent a significant route of exposure because of the extremely low rates of pollen production in potato. However, the Committee considered that the notifier should carry out case-specific monitoring to examine whether the ORF4 protein is present in the pollen of clone EH92-527-1.

While a detailed analysis of the ORFs present in the inserted DNA has been carried out, the notification does not consider the possibility of the generation of novel ORFs or the disruption of existing ORFs in the potato genomic sequence on either side of the insert site. While the Committee accepts that the detailed phenotypic characterisations presented in the notification make unexpected changes in gene expression unlikely, in order to complete the risk assessment of potato clone EH92-527-1 this information is required.

Animal feed safety

The GM subgroup of the Advisory Committee on Animal Feedingstuffs (ACAF) has concluded that, on the basis of the data provided, there is no reason to consider that feeding the by-products of starch extraction from potato clone EH92-527-1 to animals poses any additional risks to animal health compared to the parental cultivar. This conclusion is based on the thorough compositional analysis of the tubers of potato clone EH92-527-1, together with the results from a ruminant feeding study. However, the ACAF GM subgroup considered that further analysis to confirm the absence of potential unintended effects was required before their assessment could be completed. While the ACAF GM subgroup notes the arguments presented by the notifier concerning the difficulties of feeding laboratory animals raw potato material, the subgroup considers that a comparative feeding study made with rats using potato pulp derived from EH92-527-1 and from the parental cultivar is appropriate.

Completion of a study in which pulp is included to the maximum level permitted by nutritional requirements and at an intermediate level would provide a basis for concluding on the absence of any unintended effects.

ACRE agree with the assessment of the ACAF GM subgroup, and noted that the scope of the present application would not permit feeding of intact tubers from potato clone EH92-527-1 directly to animals as an alternative to processing for starch extraction.

Environmental risk assessment

ACRE considered the environmental risk assessment provided by the notifier to be thorough and agreed with its conclusions. The Committee noted the presence of the *nptII* antibiotic resistance marker gene (conferring resistance to the antibiotics kanamycin and neomycin) in potato clone EH92-527-1, but did not consider that this posed a significant risk. The *nptII* gene is widespread in bacterial populations so that its presence in a GM crop does not significantly enhance the spread of resistance to kanamycin and neomycin in bacteria. These antibiotics are not in widespread clinical or veterinary use.

The Committee noted that a strict identity preservation regime is proposed for potato clone EH92-527-1. While this regime is fit for purpose, the Committee considered that the proposed one-year potato free period following cultivation of potato clone EH92-527-1 was insufficient to guarantee the absence of volunteers of potato clone EH92-527-1 from subsequent potato crops. The Committee did not consider that the presence of potato clone EH92-527-1 in food or feed posed a risk to human or animal health, but recommended that the notifier reminds growers of their obligations to ensure that GMOs that are not authorised for human food use do not enter the food chain. This may require volunteer control measures in addition to those recommended for identity preservation purposes.

Post market monitoring

ACRE welcomed the detailed proposals for case-specific monitoring provided by the notifier. In addition to the proposed case-specific monitoring, the Committee also recommends that monitoring for the presence of the ORF4 protein in pollen is carried out (see above).

Concerning the general surveillance plan, the Committee was broadly content with the proposed approach. However, the Committee considered that additional detailed plans for general surveillance should be provided by the notifier to the satisfaction of the Swedish authorities prior to any commercial cultivation of potato clone EH92-527-1.

The Committee also noted the notifier's proposal to review the post-market monitoring plan after 5 years, with a view to extending or simplifying monitoring activities in the light of results obtained. The Committee considered that this was an appropriate approach to monitoring, which would allow the monitoring plan to be optimised in the light of experience.