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

NOTIFICATION C/GB/03/M5/3 FROM BAYER CROPSCIENCE LTD FOR CONSENT TO MARKET LLRICE62

7 January 2004

CONTENTS

1. Introduction

Bayer CropScience Ltd (Bayer) have developed rice varieties that are tolerant to the herbicide glufosinate (trade name Liberty), the transformation event for which this notification applies is known as LLRICE62 and the commercial name of the planting seed is LibertyLink rice. The product has been designated the unique identifier ACS-0S002-5. Currently there is no commercial marketing of LibertyLink rice anywhere in the world but it is expected that varieties could be available for commercial production in 2004.

No application has yet been made by Bayer to market LLRICE62 and its products under the novel food and novel food ingredients regulation 287/97. Any future application for food use would be made under the genetically modified food and feed regulation 1829/2003 which comes into effect in April 2004.

1.1. Procedure

The UK competent authority accepted this dossier on 26 August 2003. The dossier has been assessed with reference to Article 13(2) of Directive 2001/18. During the assessment period further information was requested from the applicant as follows:

- *Information on the safety of LLRICE62 to animal health.*

Study details and experimental data were requested to support the conclusion that no differences were identified for nutritive value of the rice grain and no indications of toxic or adverse effects were associated with any of the sources of rice in either of the tested animal species (poultry and swine).
Clock stopped – 27/8/03 to 1/9/03

- *Information on the expression of the insert*

In relation to Northern blot analysis, used to demonstrate the absence of RNA transcripts from the 5' and 3' flanking sequences, evidence of positive controls was requested to confirm the quality of the RNA.
Clock stopped – 24/9/03 to 14/10/03

- *Safety of the expressed PAT protein*

Clarification was requested that statements regarding the safety of the PAT protein were based on evidence from studies using the *bar* gene product. In particular data from the study in mice administered high doses of the PAT protein (encoded by *bar)* was requested. This information was required to support the conclusion that rice bran is safe when used as animal feed as the concentration of PAT protein is higher in bran than in raw grain.
Clock stopped – 13/10/03 to 3/11/03

1.2. Scientific advice

Three independent scientific committees in the UK, the Advisory Committee on Releases to the Environment (ACRE), the Advisory Committee on Animal Feedingstuffs (ACAF) and the Advisory Committee on Novel Foods and Processes (ACNFP) have considered this notification. The advice of ACRE is provided at Annex 1 and incorporates the opinion of ACAF. ACNFP had no concerns over this notification.

1.3 Public comments

The Summary Notification Information Format (SNIF) published on the Joint Research Centre website generated two representations from Members of the public originating from the UK and Holland. The second representation was 16 pages long and provided in Dutch, the UK made arrangements for this to be translated into English. Both these 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 6-39)
- Risk assessment according to Annex II of Directive 2001/18/EC (pp 40-45)
- Additional information according to Annex IV of Directive 2001/18/EC (pp 46-49)
- Monitoring plan according to Annex VII of Directive 2001/18/EC (pp 50-55)
- Appendices 1-36

2.1. Confidentiality

Bayer have claimed confidentiality for appendix 7 (LLRICE62 discriminating PCR protocol) to protect the companies 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 protocol is made public (see item 10.1).

3. Scope of the notification

The scope of this application is for the import of raw commodities containing rice grain derived from LLRICE62 and the import of processed food/feed products containing rice originating from rice grain derived from LLRICE62. This notification does not include cultivation in the EU.

4. Description of the product

The parent rice variety is Bengal, a tropical medium-grain rice developed by Louisiana State University, which has been used commercially since 1992. The intended function of the genetic modification is tolerance to glufosinate ammonium.

4.1. Transformation technique

Transformation event LLRICE62 was produced by particle bombardment also known as acceleration technology using a purified 1502bp *Hin*dIII/*Pvu*I fragment of plasmid pB5/35S*bar.* Transformed cells were selected on media containing phospinothricin.

4.2. Molecular and genetic description

4.2.1. The plasmid

The plasmid pB5/35Sbar is a derivative of pUC19 in which the β-lactamase gene has been replaced by the *nptIII* gene from pBIN19. Additionally the right border of *Agrobacterium tumefaciens* octopine plasmid pTiAch5 and the *bar* cassette have been inserted.

4.2.2. Genetic elements introduced into the GMO

The transforming 1502bp fragment is derived from plasmid pB5/35Sbar by digestion with the restriction endonucleases *Pvu*I and *Hin*dIII. This fragment contains:

- *bar* gene from the soil microorganism *Streptomyces hygroscopicus*
- 35S promoter from Cauliflower Mosaic Virus (CaMV)
- 35S terminator from Cauliflower Mosaic Virus (CaMV)

No antibiotic resistance markers are present in this restriction fragment.

4.2.3. Molecular characterisation

The molecular characterisation of LLRICE62 provided in the dossier is of sufficient quality to allow the assessment of any potential hazards. The DNA fragment used in the transformation event does not carry the *nptIII* gene or the right border sequence of the *Agrobacterium tumefaciens* octopine plasmid, pTiAch5. Information is supplied to demonstrate that these sequences are absent from LLRICE62 plants. Hence this analysis confirms that sequences from the vector backbone are absent.

Nucleotide sequence analysis has determined that the sequence of the inserted DNA is identical to the corresponding sequences in the donor plasmid vector. Southern transfer and hybridisation reveals that the transformation cassette is present as a single insert in the plant genome. Sequence analysis and comparison with sequence databases has located the site of integration in event LLRICE62 on chromosome 6 and further reveals that the transformation event caused a deletion of 18 bp in the target sequence.

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 LLRICE62 plants.

4.2.4. Gene expression

The *bar* gene (conferring bialaphos resistance) encodes the enzyme phosphinothricin-acetyl-transferase (PAT) that acetylates L-glufosinate and thereby confers tolerance to herbicides based on glufosinate ammonium.

Gene expression has been identified in the leaf, stem, root and seed of rice plants. PAT concentrations were determined by ELISA and were higher, both in absolute levels and as a percentage of total extractable protein, in rice straw (average 75.3 mg/kg fresh weight) than in rice grain (average 12.1 mg/kg fresh weight). PAT concentrations were much reduced in rice hulls

(1.6 mg/kg fresh weight) but substantially concentrated in the bran fraction (42 mg/kg fresh weight).

5. Assessment of use in animal feeds

The primary use of the rice is for human food, however rice grain and the by-products of rice milling are often included in animal diets. Rice is not normally included in the diets of farm livestock in the UK, although it may be incorporated into certain speciality pet foods. The main rice fraction used as livestock feed in the EU is rice bran mainly as a feed for ruminants. Rice (paddy) straw is extensively used as feed for ruminant animals in areas where rice is the major cereal crop. Rice straw is not a traded commodity and so consumption remains local to the areas of production.

5.1. Safety of gene products

The UK recognises that the PAT protein encoded by the *pat* gene from *Streptomyces viridochromogenes* has been examined on a number of previous occasions and deemed to be safe. The results presented in this dossier are based on experiments made with PAT encoded by the *bar* gene from *Streptomyces hygroscopicus.* These results included evidence of susceptibility to proteases in simulated gastric and intestinal models and an absence of adverse responses when the *bar* gene product was used in an acute mouse toxicity study. The experimental details provide adequate reassurance of the lack of toxicity of the *bar* gene product, even at higher concentrations found in the rice bran fraction.

5.2. Compositional analysis

Compositional data were obtained from a two-year, multi-site trial design. A total of 14 geographically distinct locations were used. At each, LLRICE62, with and without glufosinate treatment, was compared to Bengal, the parent variety, as a control.

Raw grains were analysed for their proximate composition, amino acid and fatty acid content and for a number of anti-nutritional factors. There were no significant differences found in the large majority of cases and herbicide-treatment appeared not to significantly alter the composition of the transformed rice. For a number of nutrients (including some fatty acids, iron, vitamin B1 and vitamin E), compositional equivalence was not achieved at all sites and comparisons. However, consistent trends across sites or years were not identified.

Fat, protein, ADF, NDF and ash were markedly lower in transgenic rice bran than non-transgenic bran, while total carbohydrates were higher. For fat and NDF, these exceeded the 20% bio equivalence range but remained well within the range of values reported in the literature.

The main anti-nutrients in rice are phytic acid, trypsin inhibitors and lectins. On the basis of data presented concentrations in transgenic rice appear not to differ from those seen in conventional varieties. Levels of phytic acid and trypsin inhibitor were numerically higher in the transgenic lines in both grain

and bran (phytic acid) and bran alone (trypsin inhibitor), but differences were not statistically significant. All values fell within the range reported for rice generally.

Separate comparisons were also included on brown and white rice, rice hulls, rice flour, bran oil and storage proteins. As these fractions are not commonly fed to farm animals (as distinct from companion animals) they are not further considered. However nothing in this body of data suggested that the composition of LLRICE62 rice differed in any biologically significant way from that of the control variety.

5.3. Feeding studies

5.3.1. Broiler feeding study

A total of 120 male birds (one day old) were allocated to one of two treatment groups. Both groups received starter and grower/finisher diets containing rice at a 30% inclusion level, one treatment group receiving the test article (LLRICE62) and the other a non-specified, non-transgenic control variety. The raw rice was cleaned by aspiration and sieving and then hammer-milled before inclusion into the diets. There were no significant differences ($P>0.05$) in any of the parameters measured and, within the limited capacity of the study, the results confirm the conclusion that rice event LLRICE62 is nutritionally equivalent to its non-transgenic counterpart.

5.3.2. Growing/finishing pigs

A study to evaluate the nutritional and metabolic value of the GM rice for growing/finishing pigs with a start weight of approximately 20 kg and a finish weight of approximately 100 kg was made over a 100 day period. There were four treatments – two control treatments (non-transgenic lines with one being the parent variety (Bengal) and the other a commercial long grain rice variety) and two treatment groups fed the transgenic line (one sprayed with conventional herbicide and the second with glufosinate ammonium herbicide). Twenty-four animals were used for each treatment (12 male, 12 female) assigned to six pens. Rice was incorporated into diets at between 72 – 85% depending on the age of the animal.

There were no statistically significant differences between the two non-transgenic diets and the glufosinate-treated GM for weight gain, feed:gain ratio or for all but one measure of carcass composition. The only significant difference detected ($P=0.022$) was a higher hot carcass weight in animals fed the glufosinate-treated GM grain compared to the parental control group. This appeared to relate to the numerically small but higher weight gains in the glufosinate-treated GM feed group. The feed:gain ratio was 10% lower with the conventionally sprayed GM rice compared to the mean of the other three treatments or to the glufosinate-sprayed GM rice alone ($P=0.021$). This latter observation was reflected in the final weights which were on average 7 kg higher in the glufosinate-sprayed GM group compared to the other GM group. There were no observed adverse effects of either sample of GM rice on the health of the experimental animals.

5.4. Conclusion

Overall, the compositional equivalence of the raw grain to Bengal, a variety with a close genetic background used as a control, coupled with the results of the pig and poultry studies suggests that grain and rice bran from LLRICE62 would behave as any other equivalent variety of rice and would not pose a risk to livestock or consumers of livestock products.

6. Assessment of environmental risks

In considering the environmental risk assessment for LLRICE62 the UK has restricted its assessment to only consider issues which are relevant within the scope with which the notification is made, that is for the import and processing of LLRICE62 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 have not been considered.

LLRICE62 has been compared to the non-transgenic parent variety, Bengal, for characteristics relating to plant morphology, agronomic performance, disease susceptibility, seed germination and reproductive fitness. No differences have been observed with the exception of the new characteristic, tolerance to glufosinate ammonium.

6.1. Potential gene dissemination by pollen or seed

No differences in dissemination capacity have been observed in pollen, seed and vegetative material from LLRICE62 and non-GM rice. In addition commodity rice from the USA to Europe is imported almost entirely as non-viable grain (milled rice grain). Therefore the likelihood that some imported grain could escape from silos or lorries and germinate is very low. For the purpose of this notification LLRICE62 will be imported for direct use as food and feed or for processing. No seeds will be imported for cultivation into Europe.

6.2. Potential for gene transfer

The potential for transfer of genetic material from LLRICE62 is no different to that for conventional rice varieties. Gene flow will only occur into rice crops and weed red rice 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

LLRICE 62 has no specific effects on non-target organisms when compared to non-GM rice varieties.

7. Detection method

A satisfactory PCR based protocol that is suitable for event specific detection of LLRICE62 has been provided (appendix 7 of the dossier).

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 rice grain. In order to achieve monitoring Bayer will ensure that those handling the GM rice grain are provided with information on the product and their duties within the supply chain. Surveillance will make use of existing networks e.g. national plant protection and grain inspections and national food councils. A report of the general surveillance programme will be made by Bayer 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 which alters the risk assessment be identified then Bayer 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 spilt seed is not a risk in the UK due to the fact that rice will not establish in the UK climate however other Member States may wish to consider whether the post market monitoring plan should include provisions for monitoring for spillage of seed.

9. Traceablility and labelling

Marketing of this product will be subject to the requirements of Regulation (EC) No 1829/2003 concerning traceability and labeling of genetically modified organisms and derived food and feed products. This will be a condition of consent (see item 10.1).

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 Bayer CropScience Ltd for the placing on the market of LLRICE62. The proposed consent covers the import and use for all processing and use in feed of LLRICE62 and from any progeny derived from LLRICE62 by conventional breeding methods with non-genetically modified rice. The proposed consent does not include the cultivation of LLRICE62.

10.1. Conditions of consent

The proposed consent will be granted to Bayer CropScience Ltd with the following conditions:

- For the purpose of post market monitoring the consent holder is required to submit an annual report of the outcome of general surveillance.
- In line with Article 25 (4) of Directive 2001/18/EC appendix 7of the dossier (LLRICE62 discriminating PCR protocol) must be made public.
- Reference material (seed and DNA) 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.
- Any product consisting of, or containing, LLRICE62, together with any food, food ingredient, or feed produced from LLRICE62, shall be labelled, as appropriate, in accordance with the provisions of Regulation (EC) No 1829/2003 on genetically modified food and feed and shall be subject to the traceability requirements laid down in Regulation (EC) No 1830/2203 concerning the traceability and labelling of genetically modified organisms and the traceability of food and feed products produced from genetically modified organisms and amending Directive 2001/18/EC.

ADVISORY COMMITTEE ON RELEASES TO THE ENVIRONMENT

Advice on a notification for marketing of herbicide tolerant GM rice

Notifier:	Bayer CropScience Ltd
Notification reference:	C/GB/03/M5/3
Product:	Rice genetically modified for herbicide tolerance, transformation event LLRICE62.
Scope:	For the import and use of grain varieties derived from rice transformation event LLRICE62 and processed products from rice originating from rice grain derived from LLRICE62. This notification excludes cultivation.
Date:	**25 November 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).

Advice: ACRE has considered this notification for the import and use of herbicide tolerant rice based on transformation event LLRICE62. The Committee considers that sufficient information has been provided by the notifier to demonstrate that this GM rice 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 rice 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).

ACRE recommends that reports of post-market monitoring for general surveillance of this product 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 rice. The scope of the notification excludes cultivation and the Committee considered this notification in this context. In arriving at its advice the Committee considered the notification against the requirements of the legislation as it relates to the UK.

Molecular characterisation

Transformation event LLRICE62 has been produced by particle bombardment of Bengal variety rice with a 1.5kb *Hin*dIII/*Pvu*I fragment of plasmid pB5/35S*bar.* This DNA fragment includes a copy of the *bar* gene from *Streptomyces hygroscopicus* engineered to be under the control of the Cauliflower Mosaic Virus 35S promoter and terminator sequences. Expression of the *bar* gene, which encodes the PAT protein, confers tolerance to glufosinate ammonium herbicides.

ACRE considered the molecular information provided characterising event LLRICE62 to be of sufficient quality to allow the assessment of any potential hazards. The Committee is content that the data supports the conclusion that the inserted DNA sequences are present as a single copy on chromosome 6 and that vector backbone sequences are absent from LLRICE62. Sequence analysis concludes that the insert is identical to that of the donor plasmid and the DNA flanking sequences have been identified to 149bp 5' and 670 bp 3' of the insert. Northern blot analysis was used to demonstrate the absence of RNA transcripts from the 5' and 3' flanking sequences in event LLRICE62. ACRE requested that the notifier provide evidence to confirm the quality of the RNA used in the Northern blot analysis and was satisfactorily provided.

In addition ACRE was content that the PCR detection protocol provided is event-specific.

Animal feed safety

The safety of grain derived from LLRICE62 for use as animal feed was assessed by ACAF. The main rice fraction used as livestock feed in the EU is rice bran mainly as a feed for ruminants. In considering the safety of LLRICE62 for use in animal feed the safety of the gene products, compositional analysis and data from animal feeding studies were taken into account.

The safety of the PAT protein was demonstrated through evidence of the proteins susceptibility to proteases in simulated gastric and intestinal models and an absence of adverse responses when the PAT product was used in an acute mouse toxicity study. Confirmation was requested from the notifier that these studies were conducted using the PAT protein encoded by the *bar* gene from *Streptomyces hygroscopicus.* This was satisfactorily provided.

Compositional data were obtained from a two-year, multi-site trial design. Raw grains were analysed for their proximate composition, amino acid and fatty acid content and for a number of anti-nutritional factors. There were no significant differences found and herbicide-treatment appeared not to alter the composition of the transformed rice significantly. The main anti-nutrients in rice are phytic acid, trypsin inhibitors and lectins. On the basis of data presented concentrations in transgenic rice appear not to differ significantly from those seen in conventional varieties, all values fell within the range reported for rice generally.

Separate comparisons were also conducted on brown and white rice, rice hulls, rice flour, bran oil and storage proteins. Nothing in this body of data suggested that the composition of LLRICE62 rice differed in any biologically significant way from that of the control variety.

Poultry and pig feeding studies were conducted to demonstrate the safety of LLRICE62 as animal feed. In the poultry study no significant differences in any of the parameters measured were identified and the results confirm that rice event LLRICE62 is nutritionally equivalent to its non-transgenic counterpart. It is noted that although two birds died during the experimental period, both were from causes recognised as common in broiler production. Similarly in the study in pigs no statistically significant differences were noted between two non-transgenic diets and the glufosinate-treated GM for weight gain, feed:gain ratio or for all but one measure of carcass composition, which appears to relate to the numerically small but higher weight gains in the glufosinate-treated GM feed group.

Overall, the compositional equivalence of the LLRICE62 raw grain to Bengal, a variety with a close genetic background used as a control, coupled with the results of the pig and poultry studies suggests that grain and rice bran from LLRICE62 would behave as any other equivalent variety of rice and would not pose a risk to livestock or consumers of livestock products.

Environmental risk assessment

ACRE considered carefully the environmental risk assessment for LLRICE62 provided by the notifier. The Committee noted that the genetic modification involves a well-established gene which has been present in the food chain for the last ten years without any adverse effects reported. LLRICE62 has been compared with the non-transgenic parent variety, Bengal, for characteristics relating to plant morphology, agronomic performance, disease susceptibility, seed germination and reproductive fitness. No differences have been observed with the exception of the new characteristic, tolerance to glufosinate ammonium.

ACRE considered the potential for gene dissemination and gene transfer from LLRICE62. No differences in dissemination capacity or increased potential for gene transfer have been observed in pollen, seed and vegetative material from LLRICE62 compared with non-GM rice. In addition and for the purpose of this notification LLRICE62 will be imported almost entirely as non-viable grain for direct use as processed products from rice. No seeds will be imported for cultivation into Europe. Therefore the likelihood that some imported grain could escape from silos or lorries and germinate is very low and the potential for gene transfer into rice crops and weed red rice is even lower. Members considered that because of the lack of viability of the rice seed in the UK there were no environmental risk problems. However, although the seed was unlikely to germinate in the UK there was a concern over measures to deal with accidental spillage, which could be an issue for southern European countries.

Post-market monitoring

The aim of the case-specific part of the post market monitoring plan 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 LLRICE62 there is no requirement for case-specific monitoring. For the purpose of general surveillance the notifier will make use of those people and their networks that are responsible for transport, processing and handling of the GM rice grain. Bayer have proposed to submit reports of the outcome of this monitoring 3, 6 and 10 years after authorisation. ACRE recommends that these reports be submitted annually.