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Opinion of the Scientific Panel on Genetically Modified Organisms on an application (reference EFSA-GMO-UK-2004-04) for the placing on the market of glufosinate tolerant genetically modified rice LLRICE62 for food and feed uses, import and processing, under Regulation (EC) No 1829/2003 from Bayer CropScience GmbH[1]

(No EFSA-Q-2004-145)

Opinion adopted on 30 October 2007

SUMMARY

This document provides the opinion of the Scientific Panel on Genetically Modified Organisms (GMO Panel) of the European Food Safety Authority (EFSA) on the genetically modified rice LLRICE62 (Unique Identifier ACS-OSØØ2-5), hereafter referred to as LLRICE62 developed to provide tolerance to glufosinate herbicides by expressing the PAT protein encoded by the *bar* gene.

In delivering its opinion the GMO Panel considered the application EFSA-GMO-UK-2004-04, additional information provided by the applicant (Bayer CropScience GmbH) and the scientific comments submitted by the Member States. The scope of the application is for food and feed uses, import and processing of LLRICE62 and does not include cultivation. The GMO Panel assessed LLRICE62 with reference to the intended uses and the appropriate principles described in the guidance document of the Scientific Panel on Genetically Modified Organisms for the Risk Assessment of Genetically Modified Plants and Derived Food and Feed. The scientific assessment included molecular characterization of the inserted DNA and expression of the target proteins. A comparative analysis of agronomic traits and composition was undertaken and the safety of the new proteins and the whole food/feed was evaluated with respect to nutritional quality, potential toxicity and allergenicity. An assessment of environmental impacts and the post market environmental monitoring plan were undertaken.

LLRICE62 was transformed, using particle bombardment, to express the *bar* gene, which encodes phosphinothricin acetyl-transferase (PAT). PAT acetylates glufosinate and thereby detoxifies the herbicide.

The molecular characterisation data established that LLRICE62 contains only one transgenic insert. The structure of the insert in LLRICE62 was determined by Southern analysis and DNA

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sequencing. No vector backbone sequences or antibiotic resistance marker genes were detected. Examination of the transgene locus and the plant-DNA junctions revealed 19 putative Open Reading Frame (ORF) sequences. Analysis of these ORFs was performed by bioinformatic analysis and no significant homologies were identified with known toxins or allergens or with rice genes of known function. The expression of the genes introduced by genetic modification has been sufficiently analysed and the stability of the genetic modification has been demonstrated over several generations.
The GMO Panel is of the opinion that the molecular characterisation of the DNA insert and flanking regions of LLRICE62 does not raise safety concerns, and that sufficient evidence for the stability of the insert structure was provided.

Based on the results of compositional analysis of samples from a representative range of environments and seasons, the GMO Panel concluded that both rough LLRICE62 (synonym: paddy rice, including hulls), and its processed products are compositionally equivalent to those of conventional rice, except for the presence of PAT protein. In addition, field trials did not reveal any unexpected changes in agronomic performance and phenotypic characteristics.

The PAT protein induced no adverse effects in acute and repeated dose oral toxicity studies in rodents. In addition, PAT is rapidly degraded in simulated gastric fluid and inactivated during heat treatments.

A 42-day feeding study on broilers and a 96-day study on growing-finishing pigs did not indicate differences in the nutritional value of LLRICE62 compared with the non-GM comparator. These animal studies support the compositional analysis which indicated no unintended effects.

The application EFSA-GMO-UK-2004-04 concerns food and feed uses, import and processing of LLRICE62. There is therefore no requirement for scientific information on possible environmental effects associated with cultivation. Accidental release of viable GM paddy rice into the environment is possible and GM seeds could be dispersed into land cultivating rice and establish GM populations, which could outcross with non-GM cultivated or weedy rice plants. The GMO Panel concluded that there is a possibility that small numbers of GM rice plants could enter cultivation and cross-pollinate with cultivated or weedy rice. However it is unlikely that spillage will result in feral plants establishing around ports, mills, and transit routes as there is no indication of changes in fitness or behaviour of this GM rice, except in the presence of glufosinate.

The GMO Panel advised that appropriate management systems should be in place to prevent seeds of LLRICE62 entering cultivation. The monitoring plan provided by the applicant is in line with the intended uses of LLRICE62. Furthermore the GMO Panel agrees with the reporting intervals proposed by the applicant in the general surveillance plan.

In conclusion, the GMO Panel considers that the information available for LLRICE62 addresses the scientific comments raised by the Member States and that LLRICE62 is as safe as its non-GM comparator with respect to potential effects on human and animal health or the environment. Therefore the GMO Panel concludes that LLRICE62 is unlikely to have any adverse effect on human and animal health or on the environment in the context of its intended uses.

Key words: GMO, rice, *Oryza sativa*, ACS-OSØØ2-5, PAT protein, *bar* gene, herbicide tolerant, glufosinate, food and feed safety, human and animal health, environment, import, food, feed, Regulation (EC) 1829/2003, Directive 2001/18/EC.

TABLE OF CONTENTS

BACKGROUND

On 20 August 2004 EFSA received from the United Kingdom Competent Authority (Food Standards Agency) an application (Reference EFSA-GMO-UK-2004-04), for authorisation of the herbicide-tolerant genetically modified rice LLRICE62 (Unique Identifier ACS-OSØØ2-5), hereafter referred to as LLRICE62, submitted by Bayer CropScience GmbH within the framework of Regulation (EC) No 1829/2003 on genetically modified (GM) food and feed (EC, 2003) for food and feed uses, import and processing. On 24 September 2004 EFSA was asked to provide an opinion on notification C/GB/03/M5/3 on LLRICE62, submitted under the Directive 2001/18/EC (EC, 2001). However, the applicant withdraw this notification, as it was covered by the application EFSA-GMO-UK-2004-04, under Regulation (EC) No 1829/2003 and consequently EFSA was no longer required to issue an opinion under the Directive 2001/18/EC on LLRICE62.

After receiving the application EFSA-GMO-UK-2004-04 and in accordance with Articles 5(2)(b) and 17(2)b of Regulation (EC) No 1829/2003, EFSA informed the Member States and the European Commission and made the summary of the dossier available to the public on the EFSA website. EFSA initiated a formal review of the application immediately, to check compliance of the dossier with the requirements laid down in Articles 5(3) and 17(3) of Regulation (EC) No 1829/2003. On 14 January 2005 EFSA declared the application as valid and started the clock in accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003.

EFSA made the valid application available to Member States and the European Commission and consulted nominated risk assessment bodies of the Member States, including the national Competent Authorities within the meaning of Directive 2001/18/EC (EC, 2001) following the requirements of Articles 6(4) and 18(4) of Regulation (EC) No 1829/2003, to request their scientific opinion. The Member State bodies had three months after the date of receipt of the valid application (until 14 April 2005) within which to make their scientific comments known.

The GMO Panel carried out a scientific assessment of LLRICE62 taking account of the appropriate principles described in the Guidance document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants and derived food and feed (EFSA, 2006a).

On 21 March 2005, 12 July 2005, 5 June 2007, 18 June and 21 September 2007 the GMO Panel asked the applicant for additional data or clarifications on LLRICE62. The applicant provided the requested information on 10 May 2005, 29 March 2007, 7 June 2007, 4 July 2007

and 11 October 2007 respectively. After receipt and assessment of the full data package, the GMO Panel finalized its risk assessment on LLRICE62.

The GMO Panel carried out the scientific assessment of the genetically modified LLRICE62 for food and feed uses, import and processing in accordance with Articles 6(6) and 18(6) of Regulation (EC) No 1829/2003, taking into consideration the scientific comments of the Member States and the additional information provided by the applicant.
In giving its opinion on LLRICE62 to the European Commission, the Member States and the applicant, and in accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003 EFSA has endeavoured to respect a time limit of six months from the receipt of the valid application. As additional information was requested by the GMO Panel, the time limit of 6 months was extended accordingly, in line with Articles 6(1), 6(2), 18(1), and 18(2) of Regulation (EC) No 1829/2003.

According to Regulation (EC) No 1829/2003, the EFSA opinion shall include a report describing the assessment of the food and feed and stating the reasons for its opinion and the information on which its opinion is based. This document is to be seen as the report requested under Articles 6(6) and 18(6) of that Regulation and thus will be part of the overall opinion in accordance with Articles 6(5) and 18(5).

TERMS OF REFERENCE

The GMO Panel was requested to carry out a scientific assessment of the genetically modified LLRICE62 for food and feed uses, import and processing in accordance with Articles 6(6) and 18(6) of Regulation (EC) No 1829/2003. Where applicable, any conditions or restrictions which should be imposed on the placing on the market and/or specific conditions or restrictions for use and handling, including post-market monitoring requirements based on the outcome of the risk assessment and, in the case of GMOs or food/feed containing or consisting of GMOs, conditions for the protection of particular ecosystems/environment and/or geographical areas should be indicated in accordance with Articles 6(5)(e) and 18(5)e of Regulation (EC) No 1829/2003.

The GMO Panel was not requested to give an opinion on information required under Annex II to the Cartagena Protocol. Moreover, the GMO Panel did also not consider proposals for labelling and methods of detection (including sampling and the identification of the specific transformation event in the food/feed and/or food/feed produced from it), which are matters related to risk management.

ASSESSMENT

1. Introduction

LLRICE62 was assessed with reference to its intended use and the appropriate principles described in the 'Guidance Document of the Scientific Panel on Genetically Modified Organisms for the Risk Assessment of Genetically Modified Plants and Derived Food and Feed' (EFSA, 2006a). In its evaluation the Panel also considered the issues that were raised

by Member States during the initial assessment of the application introduced under Regulation No 1829/2003. The assessment presented here is based on the information provided in the application including additional information from the applicant in reply to GMO Panel questions and Member State comments.

2. Molecular characterisation

2.1 Issues raised by the Member States

Member States raised questions concerning a) the number of generations used for stability testing by Southern analyses; b) the interruption of an endogenous gene of unknown function by the transgenic insert and the need to characterise the gene family; c) the need for more testing of insert expression including expression of potential new proteins.

2.2 Evaluation of relevant scientific data

2.2.1 Transformation process and vector constructs

The vector used for the transformation, pB5/35S*bar*, is a derivative of the vector pUC19 in which the β-lactamase gene was replaced by the *npt*III gene from vector pBIN19. The resulting plasmid was further modified by insertion of a DNA element containing the right border of the *Agrobacterium tumefaciens* octopine plasmid pTiACH5 into the single *Nde*I site of the vector. There is no left border sequence present in the vector. No carrier DNA was used in the transformation process.

The 1501 bp *Hind*III/*Pvu*I fragment of plasmid pB5/35S*bar*, used in the transformation, contains one open reading frame, the *bar* gene, which encodes phosphinothricin acetyl-transferase (PAT), that acetylates glufosinate ammonium and thereby detoxifies the herbicide. The expression of *bar* is regulated by the 35S CaMV promoter and the 35S CaMV terminator, derived from the cauliflower mosaic virus. The *bar* gene itself was isolated from genomic DNA of *Streptomyces hygroscopicus*, a common soil microbe, not known to be a human, animal or plant pathogen.

Rice tissue used for transformation was obtained from embryos derived from surface sterilized seeds of variety Bengal. The DNA preparation and delivery via particle bombardment were performed following established methods. Transformed cells were selected on media containing glufosinate ammonium, and allowed to develop into transgenic callus. This callus was transferred to regeneration medium, which induces shoot and root development. The plantlets that developed were transferred to the greenhouse, and allowed to flower and set seed.

2.2.2 Transgenic constructs in the genetically modified plant

The putative size of the DNA fragment used for transformation was originally determined to be 1502 bp. However, sequence analysis subsequently showed the size of the fragment used for transformation to be 1501 bp. The length of the sequence actually inserted into LLRICE62 was shown to be 1497 bp. This 5 bp length difference between the putative plasmid sequences and the integrated sequence is the result of a 1 bp deletion and the non-integration of a 4 bp *Hind*III overhang. The observed single base pair deletion occurred in a non-coding region of the

pB5/35Sbar plasmid. This part of the LLRICE62 insert sequence was shown to be completely identical to the corresponding transforming plasmid sequence.

Southern analysis of genomic DNA from transformation event LLRICE62 included digestions with *Hin*dIII, *Eco*RV, *Bgl*II and *Sac*II enzymes and probes for 35S promoter, *bar* and 35S terminator. The probes used covered the entire T-DNA. Southern analysis data indicate a single copy of the transgenic insert (which is located on chromosome 6 of the rice genome). However, Southern analysis of *Nco*I and *Eco*RI digested genomic LLRICE62 DNA showed the presence of unexpected fragments. The hypothesis that these arose from incomplete DNA digestion was assessed by Southern analysis, PCR and sequence analysis. Incomplete digestion of DNA was observed even following an overnight incubation of LLRICE62 DNA with *Eco*RI and *Nco*I. To complement the Southern analysis the complete LLRICE62 transgenic locus was reconstructed, based on the determined sequences of the locus and use of wild-type rice DNA sequences from databases. For the verification of the complete LLRICE62 transgenic locus sequence, genomic LLRICE62 DNA was subjected to PCR analysis, using a series of primers in the 5' flanking sequences (obtained from the *in silico* analysis) together with an insert-specific primer. This study revealed significant PCR amplification problems caused by the presence of secondary structures and a high GC percentage present in the 5' flanking sequences. These 5' flanking sequences correspond to a repeated sequence, identified as MERMITE18D. The data therefore support the hypothesis that the unexpected DNA fragments observed in Southern blots of LLRICE62 DNA after digestion specifically with *Nco*I and *Eco*RI, arise from incomplete DNA digestion. The evidence therefore supports the conclusion that there is only one copy of the transgenic locus.

Southern analyses also demonstrated the absence of vector backbone and antibiotic resistance marker genes.

Examination of the transgene locus and the plant-DNA junctions revealed 19 putative Open Reading Frame (ORF) sequences. An up-to-date bioinformatic analysis was completed on these ORFs in September 2007, evaluating the amino acid (aa) sequence homology between the ORFs and proteins contained in the most up- to- date Uniprot-Swissprot, Uniprot-trEMBL, PIR, DAD, Nrl-3d, GenPept, and Allergen databases. The analysis used FindPatterns or BLASTP algorithms. Short ORF sequences (less than 8 aa) were not analysed and these included ORF-1 (5 aa), ORF-2 (6 aa), ORF-3 (6 aa), ORF-4 (4 aa), ORF-6 (4 aa), ORF-7 (5 aa), ORF 10 (5 aa), and ORF-14 (4 aa). The results of *in silico* analysis of the putative ORF-5, ORF-8, ORF-9, ORF-11, ORF-12, ORF-13, ORF-16, ORF-17, ORF-18, ORF-19 sequences showed no relevant identities with known proteins in the databases, including toxins or allergens. No homologies were identified between these ORF sequences and rice proteins. When performing a BLASTn search using the rice specific EST and mRNA databases, ORF-5 showed sequence similarity with the 5' end of rice mRNA. Similarities were observed with several mRNAs or cDNA sequences. Multiple alignment analysis revealed high homologies, but not identities, between eight different cDNA clones. The cDNA clones therefore probably belong to the same gene family, which is consistent with studies which revealed that the *bar* gene cassette of LLRICE62 was inserted into a repeated element. Although ORF-5 did show sequence similarities with rice mRNA and with two hypothetical rice proteins, no specific function could be ascribed to these putative proteins. Furthermore, bioinformatic analysis revealed no homology with a ribosome binding site or with core promoter sequences. This indicates that ORF-5 is not transcribed and this was confirmed by Northern analysis. Similarly, whilst the protein homology search identified hits with ORF-9, sequence similarities with the putative protein of unknown function were extremely low (10 amino acids for a total of 730). Thus no sequence homology with known functional rice proteins was found for the predicted cryptic ORFs, using current databases.

Significant amino acid sequence homologies were identified between the putative ORF-15 sequence and proteins in the databases. ORF-15 is located on the 35S cauliflower mosaic virus (CaMV) promoter sequence, an integral part of the *bar* gene insert. No identities were found between ORF-15 and rice proteins and ORF-15 sequence has no similarities with known allergens present in the allergen database. Furthermore, Northern analysis has demonstrated that no mRNA is transcribed from ORF-15, and thus no protein encoded by ORF-15 is actually expressed in LLRICE62.

Only ORF-12, located at the 3' end of the insert, shows homology with rice cDNA sequences. However the region of homology corresponds to the cloning vector of all these cDNA clones. When performing a multiple alignment a perfect match of 123 nucleotides was observed between ORF-12 and ten cDNA clones. However, this nucleotide sequence corresponds to the part of ORF-12 already reported as originating from the multiple cloning site of pUC19 (modified LacZ gene). This indicates that all these reported cDNA sequences still contain part of the plasmid sequence into which these cDNAs were cloned. Therefore, the homology between ORF-12 and rice mRNA is an artifact. Indeed, no hits with rice ESTs or mRNAs were found when a homology search was carried out using only that part of ORF-12 originating from the rice flanking sequence.

Based on the analyses provided the putative ORF amino acid sequences identified from LLRICE62 do not present any significant sequence identity with known toxins and allergens or with rice genes of known function.

2.2.3 Information on the expression of the insert

Expression of the *bar* gene was demonstrated in leaf, stem, root and seed of LLRICE62. The PAT protein constituted 11.9 to 12.7 µg/g fresh weight (FW) of roots, 30.4 to 30.9 µg/g FW of stems (culm) and 81.7 to 99.8 µg/g FW weight of leaves. PAT protein comprised an average of 0.23, 0.19 and 0.13% of the total crude protein in roots, stems and leaves, respectively. PAT protein constitutes 12.1 µg/g FW of grain and 75.3 µg/g FW of straw. Taking into account the moisture content of the respective grain samples and straw samples, the PAT protein contents on a dry matter basis are, accordingly, 15.1 µg/g dry weight of grain and 192 µg/g dry weight of straw. Using the values for the amount of crude protein in these fractions, PAT protein comprises 0.02% and 0.32% of the protein in grain and straw of LLRICE62, respectively. PAT protein was not found in any of the non-transgenic controls. The limit of detection is 2.82 ng/g FW for the grain and 2.63 ng/g FW for the straw.

A part of the 3' flanking sequence showed sequence similarity (96%) with *Lupinus luteus* transfer RNA^{Leu} ($tRNA^{Leu}$) gene. Based on *in silico* analysis the presence of a homologous tRNA gene in the 3' flanking sequence of LLRICE62 located 403 bp downstream from the integration site was predicted. Northern analysis showed that the predicted tRNA gene is active in all leaf, stem, root and seed tissues of wild type rice and in event LLRICE62 plants.

2.2.4 Inheritance and stability of inserted DNA

The stability of the transformation event in LLRICE62 was determined by segregation analysis of the trait and by Southern analysis. Based on selection for the PAT phenotype (herbicide tolerance), segregation analysis indicated Mendelian inheritance of a simple dominant trait. Southern analyses included the use of T2, T3, T5 and T6 LLRICE62 generations and several

growing locations including Puerto Rico and, in the USA, Louisiana, and Texas. The data from *Eco*RV DNA digests probed with either the *bar* cassette (1501 bp *Hind*III-*Pvu*I) fragment or the pB5/35S*bar* vector indicated stability of the transgenic locus, verified the structure of the insert and demonstrated the absence of vector sequences. To further demonstrate the stability of event LLRICE62, rice variety Bengal containing the LLRICE62 event was crossed with several individual plants representing four rice varieties with distinct genetic backgrounds. Again, probing *Eco*RV restricted genomic DNA from event LLRICE62 with the *bar* gene cassette showed the two expected bands in all test samples. These bands correspond to the junctions between transgenic sequences and plant DNA sequences upstream and downstream of the insert and were identical in all sample plants from four different genetic backgrounds.

2.3 Conclusion

The molecular analysis of the LLRICE62 event indicates the presence of only one copy of the transgenic insert. Examination of the transgenic locus and the plant-DNA junctions revealed 19 putative Open Reading Frame (ORF) sequences. An up-to-date (September 2007) bioinformatic analysis was carried out on these ORFs, evaluating amino acid sequence homologies with proteins contained in the most up- to-date databases. The putative ORFs had no significant sequence homologies with known toxins or allergens. Similarly, no sequence homology with known functional rice proteins was found for the predicted cryptic ORFs.

Based on selection for the PAT phenotype (herbicide tolerance), segregation analysis indicated Mendelian inheritance of a simple dominant trait. Southern analyses verified the structure and stability of the transgenic locus over several generations.

Expression of PAT was demonstrated in leaf, stem, root and seed of LLRICE62. The levels of protein accumulated do not indicate any safety concern. The GMO Panel considers the molecular analyses sufficient and that no safety concerns are evident.

3. Comparative analysis

3.1 Issues raised by Member States

Questions were raised regarding a) the assessment of compositional differences between LLRICE62 and the conventional comparator; b) the design of the field trials to produce material for compositional analysis; c) the need for additional field trials after taking into account the outcomes of compositional analyses which had been submitted in the original dossier and d) the statistical analysis of the compositional data.

3.2 Evaluation of relevant scientific data

Having considered the information provided in the application and the Member States comments, the GMO Panel requested from the applicant further analysis of the compositional data with respect to the statistical treatment (ANOVA) and additional field trials using a randomised replicated block design covering more than one growing season, including plots with LLRICE62 treated with glufosinate. The applicant performed the requested field trials and the rough rice produced was subjected to compositional analyses. The data obtained were

analysed using statistical approaches which were in line with the recommendations of the GMO Panel.

3.2.1 Choice of comparator and production of material for the compositional assessment

For compositional studies, LLRICE62 was compared to the non-transgenic parental variety Bengal, which was accepted by the GMO Panel as the most appropriate conventional comparator. The field trials utilised for comparative purposes were carried out during the years 1998, 1999, 2005, and 2006 at various locations in the US and in Canada. The field studies that were performed in 1999 and 2005 were located in the same five regions, using a randomised replicated block design, to compare LLRICE62 and its non-GM comparator treated with conventional herbicides. Moreover, the trial from 2006 included conventional rice varieties Cocodrie, Francis, and Cherniere to allow a wider comparison of any compositional differences in LLRICE62. The Panel considered that the data provided from this material were in accordance with the requirements of the guidance document (EFSA 2006a).
In addition, in the 2006 growing season, data from LLRICE62 treated with glufosinate or conventional herbicides and its non-GM comparator treated with conventional herbicides were collected.

Rice products consumed as food comprise brown rice obtained by dehulling of rough rice (synonym: paddy rice, including hulls), as well as various downstream products, *e.g.* white rice, parboiled rice, rice bran, and rice bran oil. Rice products for animal nutrition comprise rough rice and derived feed materials as well as rice straw. Therefore rough rice and rice straw were accepted as appropriate matrices for comparative compositional analysis of food and feed derived from LLRICE62. The GMO Panel also considered data provided by the applicant from technologically processed rice products, *i.e.* brown rice, polished (white) rice, rice bran, rice flour, crude rice bran oil and parboiled rice.

3.2.2 Compositional analysis

Rough rice was collected during field trials for compositional analysis. The compositional analysis of LLRICE62 and its non-transgenic comparator Bengal was carried out with respect to proximates, fibre compounds, micro-nutrients (minerals, vitamins), amino acids, fatty acids, and anti-nutrients (*i.e.* phytic acid, trypsin inhibitors, lectins). The set of food constituents analysed was in agreement with the key nutrients, and anti-nutrients recommended by OECD (OECD, 2004). An additional analysis (ANOVA) of compositional parameters was provided by the applicant in response to a request by the GMO Panel. ANOVA analysis of the compositional data obtained from individual locations showed statistically significant differences in the level of several compounds. However, when these differences from the five regions in each of the years (1999 and 2005) were examined, the endpoints showed no statistically significant differences which were consistent in both years. In addition, it was demonstrated that the observed differences were within the ranges determined for the commercial varieties and in agreement with the natural variability as reported in the literature (OECD, 2004; Poulsen *et al.*, 2007a; Poulsen *et al.*, 2007b; Schroder *et al.*, 2007).

In addition to the analysis of rough rice the applicant analysed rice straw for proximates as well as various processed rice products, *i.e.* brown rice, polished (white) rice, rice bran, rice flour, crude rice bran oil and parboiled rice for additional parameters, *e.g.* Osborne protein fractions in brown rice, oryzanol and vitamin E in rice bran oil. Compositional equivalence between

LLRICE62 and its corresponding control was confirmed in the analysis of processed food and feed commodities.

The GMO Panel considered the observed compositional differences between LLRICE62 and its comparator in the light of the field trial design, measured biological variation and the level of the studied compounds in commercial rice varieties, and concluded that LLRICE62 is compositionally equivalent to the non-GM comparator as well as other conventional rice varieties, except for the introduced trait.

3.2.3 Agronomic traits and GM phenotype

The applicant provided information on agronomic performance and phenotypic characteristics of LLRICE62 and its non-GM comparator in the course of field trials performed at several locations over up to 4 growing seasons. Replicated agronomic evaluations were conducted in 1999, 2000 and 2001 in the US. Additional field studies were conducted in Brazil and Argentina in the 1998-1999 and 1999-2000 growing seasons. Amongst others, the following agronomic parameters were tested: plant morphology, grain characteristics, agronomic performance, disease susceptibility, seed germination, and reproductive fitness. The GM LLRICE62 and its non-GM comparator were shown to be similar in the following characteristics: yield, culm length, panicle length, days to heading, days to maturity, grain shape. The LLRICE62 plant was shorter than its non-GM comparator, but still within the ranges determined for the whole spectrum of commercial varieties. The GMO Panel does not consider that this raises any food and feed safety concern. There were no observable differences in field resistance to *Drechslera oryzae* and *Rhyncosporium oryzae* between LLRICE62 and its non-GM comparator.

The GMO Panel assessed the data provided and considers LLRICE62 to be agronomically equivalent to the currently grown non-GM variety Bengal, with the exception of the newly introduced trait and the reduction in plant height.

3.3 Conclusion

Analyses carried out on materials from LLRICE62 and its closely related comparator Bengal indicated that food and feed derived from LLRICE62 and from the conventional comparator are compositionally equivalent except for the introduced trait. The comparative analysis of LLRICE62 provided no indication of unintended effects resulting from the genetic modification.

4. Food/feed safety assessment

4.1 Issues raised by Member States

Questions were raised regarding the need for further animal feeding studies, such as a 90 day subchronic toxicity study in rats, further feeding studies with ruminants and fish, the use of *E. coli* produced PAT/*bar* protein in the safety studies. To assess potential allergenicity the use of a threshold of 6 contiguous amino acids of PAT was proposed instead of 8 amino acids in the bioinformatic studies used.

4.2 Evaluation of relevant scientific data

4.2.1 Product description and intended use

The scope of application EFSA–GMO-UK-2004-04 covers the import and processing of LLRICE62 and its derived products for food and feed uses *e.g* polished rice, parboiled brown rice, milled rice, rice bran, and rice bran oil.

The genetic modification is intended to improve agronomic performance only and does not influence the nutritional quality, production processes and overall use of rice as a crop. This application does not include cultivation of LLRICE62 within EU.

4.2.2 Effects of processing

LLRICE62 has been found to be compositionally equivalent to the conventional rice used as the non-GM comparator except for the newly expressed trait (see Section 3.2.2). The effect of temperature on recombinant PAT protein produced by *E. coli* was assessed by SDS-PAGE following incubation for up to 60 minutes at 60, 75 and 90°C. This did not indicate any degradation of the PAT protein. However, Western blots detected smaller fragments in addition to the main PAT protein band after heating for 15 minutes at temperatures between 80°-100°C indicating partial degradation at these temperatures. Nevertheless, PAT activity was lost at temperatures at 60°C and higher (Wehrmann et al, 1996).

PAT protein levels were assessed by ELISA in matrices such as rice hulls, brown rice, parboiled brown rice, polished rice, rice bran, rice flour and rice bran oil. The PAT protein content expressed as a %(w/w) of crude protein ranged from 0.0065% in rice hulls to 0.0164% in rice flour. No PAT protein was detected in bran oil and parboiled brown rice.

Taking into account the compositional analysis (section 3.2.2) the Panel has no reason to assume that the processing characteristics of rice products derived from LLRICE62 would be different from those of non-GM processed rice.

4.2.3 Toxicology

4.2.3.1 PAT protein used for the safety assessment

The PAT protein in LLRICE62 is encoded by the *bar* gene from *Streptomyces hygroscopicus*. Due to the low expression level of the PAT protein in LLRICE62 and the very difficult task of isolating a sufficient quantity of purified plant protein, protein safety studies were conducted with a PAT protein encoded by the *bar* gene (PAT/*bar* protein) and expressed in *E. coli*. The *E. coli* produced PAT protein differs from the LLRICE62 plant PAT protein by a single amino acid at the penultimate position from the N-terminus where an aspartic acid has replaced the serine occurring in the plant protein. However, the similarity of the PAT proteins produced by *E. coli* and plant was demonstrated by N-terminal amino acid sequencing using Edman degradation, immunoreactivities in Western analysis, lack of post–translational modification (glycosylation) as indicated by mobility in SDS-PAGE, mass spectrometry, and enzymatic activity. Based on the identified similarity in structure and function between these proteins, the GMO Panel accepts the use of PAT/*bar* protein derived from *E. coli* for the safety testing of the PAT protein present in LLRICE62.

4.2.3.2 Toxicological assessment of expressed novel proteins

(a) Acute toxicity and repeated dose oral toxicity study

The applicant provided a single dose acute toxicity study in mice with PAT/*bar* protein produced in *E.coli*. The potential toxicity of the PAT protein was studied after intravenous injection of the protein at dose levels of 1 and 10mg/kg body weight. The intravenous injection was chosen because of the expected fast proteolytic degradation of the protein in digestive environments. No mortality and no treatment related signs of systemic toxicity were observed during the 14-day observation period even at the high dose of 10mg/kg body weight.

In a repeated dose oral toxicity study over 14 days, PAT protein, encoded by the *pat* gene from *Streptomyces viridochromogenes* (PAT/*pat)* and produced in *E.coli*, was administered in the diet to Wistar rats at the concentrations of 5,000 and 50,000 mg/kg diet. Control rats received either a standard rat diet, or a standard diet supplemented with 50,000 mg of soya protein /kg of diet. The study comprised 4 groups of rats with 5 females and 5 males per group. No mortality was observed in this study. Several statistically significant differences between the test and control groups were observed in the clinical biochemistry parameters. Total cholesterol and phospholipid levels were increased ($p<0,05$) in males fed diets containing 5,000 and 50,000 mg of PAT protein/kg diet. In females, increased phospholipid levels ($p<0,01$) were observed in the group receiving a diet containing 50,000 mg of PAT protein/kg diet. As these findings also occurred in animals fed a diet containing 50,000 mg of soya protein/kg diet, they are not attributable to administration of the PAT protein. There were no differences in organ weights and macroscopic and microscopic parameters investigated. Thus, the repeated dose oral toxicity study with the PAT protein encoded by the *pat* gene showed no evidence of toxicity.

As the PAT/*pat* and PAT/*bar* proteins have been shown to be structurally and functionally similar (Wehrmann et al, 1996; Herouet et al, 2005), the Panel accepts the use of the PAT/*pat* protein for the safety assessment of the PAT/*bar* protein in LLRICE62.

(b) Degradation in simulated digestive fluids

The stability of the PAT/*bar* protein, produced in *E.coli*, was tested in *in vitro* digestibility studies.

The bacterially produced PAT/*bar* protein was degraded within 30 sec at pH 2, when incubated in simulated gastric fluid containing pepsin. Degradation was demonstrated by Coomassie blue staining of proteins following SDS–PAGE. Degradation of the PAT/*bar* protein was also demonstrated by Western blots after 5 minutes of incubation in simulated intestinal fluid at pH 7.5 containing pancreatin.

The *in vitro* digestion experiments demonstrate that the PAT/*bar* protein is rapidly degraded in simulated gastric and intestinal fluids.

(c) Bioinformatic studies

Searches for amino acid sequence homology of the PAT protein in LLRICE62 with sequences from protein databases indicated significant homology only with other acetyltransferases. There was no sequence homology with known toxic proteins.

On the request from the Panel the applicant provided an updated bioinformatic comparison of putative ORFs in LLRICE62 with sequences of known toxins or known allergens contained in

updated publicly available databases. This bioinformatic analysis showed no significant homology with known toxic proteins and allergens (see section 2.2.2).

4.2.3.3 Toxicological assessment of new constituents other than proteins

No new constituent other than the PAT protein is expressed in LLRICE62 and no relevant changes in composition were detected by compositional analysis.

4.2.4 Toxicological assessment of the whole GM food/feed

No oral toxicity studies with the whole GM food/feed were provided.

The GMO Panel after considering all the data available on the molecular characterization, compositional analysis and agronomic performance, came to the conclusion that LLRICE62 is equivalent to its non-GM comparators, except for the introduced trait. Therefore, the Panel did not see the need for further animal safety studies with the whole food/feed.

4.2.5 Allergenicity

The strategies used when assessing the potential allergenic risk focus on the characterisation of the source of the recombinant protein, the potential of the newly expressed protein to induce sensitisation or to elicit allergic reactions in already sensitised persons and whether the transformation event may have altered the allergenic properties of the modified food. A weight-of-evidence approach is recommended, taking into account all of the information obtained with various test methods, since no single experimental method yields decisive evidence for allergenicity (CAC, 2003; EFSA, 2006a).

4.2.5.1 Assessment of allergenicity of the newly expressed proteins

The *bar* gene originates from *Streptomyces hygroscopicus*, a soil organism that is not known to be allergenic.

The sequence of the PAT protein as expressed in LLRICE62 was subjected to bioinformatic analysis. A search using 80 amino acid window indicated no similarity with known allergenic proteins when applying a 35 % identity criterion. The results of a sequence homology search to identify sequences of at least 8 contiguous amino acids showed no similarities between known allergens and the PAT protein expressed by LLRICE62. In addition, in a published bioinformatic study no amino acid sequence similarity between short stretches (i.e 6 amino acids) of the PAT protein and short stretches of known allergens were identified (Kleter and Peijnenburg, 2002). The PAT protein has a high sequence similarity only with other acetyltransferase. It is not known to be allergenic, is not glycosylated and is not stable in simulated gastric and intestinal fluids (Herouet, 2005). Based on these results the GMO Panel considers that the newly expressed PAT protein is not likely to be allergenic.

4.2.5.2 Assessment of allergenicity of the whole GM plant

Allergenicity of the whole crop could be increased as an unintended effect of the random insertion of the transgene in the genome of the recipient, for example through qualitative or

quantitative modifications of the pattern of expression of endogenous proteins. RAST inhibition and immunoblotting studies with LLRICE62 based on 15 individual rice allergic patients showed that the allergenic potential of the genetically modified rice does not differ from its non-GM comparator. Moreover an ELISA-based test using antibodies specifically recognising 14-16kDa rice allergenic proteins detected 0.415 mg/g dry weight from flour of rice kernels in conventional rice and 0.304 mg/g in the LLRICE62. The GMO Panel concludes that the information presented confirms that the overall allergenicity of the whole plant is not changed.

4.2.6 Nutritional assessment of GM food/feed

LLRICE62 is compositionally equivalent to its conventional comparator and to other commercially available rice except for the presence of the newly expressed protein. Therefore, the GMO Panel does not require additional feeding/nutritional studies. However, two feeding studies were performed. One of these was a 42-day feeding study in male broiler chickens. Two groups of 60 broilers were fed diets containing 30% rice kernels, one of the groups LLRICE62 and the other the non-GM comparator. There was no significant difference in feed intake, feed conversion efficiency, weight gain or carcass quality parameters between the two treatment groups.

The other feeding study was a 96-day study of growing-finishing pigs. In this study, the pigs received diets containing about 80% of kernels that were supplemented with soybean meal and appropriate amino acids. Four groups of 24 pigs received a diet containing kernels from LLRICE62 treated with conventional herbicides, LLRICE62 treated with glufosinate, the non-GM control rice variety treated with conventional herbicides and a commercial rice variety treated with conventional herbicides. Effects on weight gain, feed conversion efficiencies and carcass quality parameters were determined. No adverse performance effects of feeding LLRICE62 were observed.

Significant differences were found only in the hot weight at study termination and in the weight gain in the middle phase of the study between the two control groups and the group of pigs fed LLRICE62 treated with glufosinate. However, there were no statistically significant differences between the control groups and the group of pigs fed LLRICE62 treated with glufosinate in total weight gain at sacrifice, feed conversion efficiency, in 10th rib backfat thickness, in 10th rib longissimus area thickness, in carcass lean percentage or the dressing (*i.e.* the difference in weight before and after slaughter expressed in % of weight before slaughter).

There were also significant differences in total weight gain, feed conversion efficiency and the hot weight at study termination between the group of pigs fed LLRICE62 treated with conventional herbicides and group of pigs fed LLRICE62 treated with glufosinate. These differences were however found only between the herbicides treatments and that the compositional analysis of the GM and non-GM rice show similarity in constituents. The feeding studies with poultry and pigs reported in the dossier are relevant for the nutritional assessment of the LLRICE62 and the Panel considers that further feeding studies in other animal species are not necessary.

4.2.7 Post-market monitoring of GM food/feed

The risk assessment concluded that no data have emerged to indicate that LLRICE62 is any less safe than its non-GM comparator. In addition, rice LLRICE62 is, from a nutritional point of view, substantially equivalent to conventional rice. Therefore, and in line with the Guidance document

(EFSA, 2006a), the GMO Panel is of the opinion that post-market monitoring of the GM food/feed is not necessary.

4.3 Conclusion

No toxicity of the PAT protein was observed in an acute toxicity study in mice after intravenous injection and in repeated dose oral toxicity study over 14 days in Wistar rats. The PAT protein is rapidly degraded in simulated gastric and intestinal fluids. The PAT shows no homology with known toxic proteins and/or allergens. Furthermore, the PAT has been extensively assessed in previous opinions of the EFSA GMO Panel and found to be safe (EFSA 2004, EFSA2005a,b,c,d, EFSA2006c, EFSA 2007). No issues were raised concerning the safety of the PAT protein. An extensive comparative compositional and agronomical analysis showed that LLRICE62 is equivalent to conventional rice except for the introduced trait. A 42-day feeding study with broiler chickens did not provide any indications that LLRICE62 and its non-GM comparator are nutritionally different. No relevant performance effects were observed in a 96-day feeding study with growing finishing pigs. The GMO Panel is of the opinion that the LLRICE62 is as safe as conventional rice and that the overall allergenicity of the whole plant is not changed due to genetic modification. The GMO Panel considers that no additional animal safety or nutritional study is needed.

5. Environmental risk assessment and monitoring plan

5.1 Issues raised by the Member States

Comments were given regarding the occurrence of cross-compatible rice relatives in Europe and the potential for outcrossing between imported GM rice and cultivated, weedy and/or feral rice.

Further comments were raised regarding the herbicide use, the need for more detailed environmental post-market monitoring measures as well as for specific management measures to avoid accidental release of LLRICE62 into the environment.

5.2 Evaluation of relevant scientific data

5.2.1 Environmental risk assessment

Rice, *Oryza sativa* L., was originally introduced from Asia as both a cultivated and weedy form and is the main rice species present in Europe as both cultivated types and weedy rice including red rice. In addition hybridisation between cultivated and weedy types has resulted in some intermediate types. Rice is currently cultivated in eight Member States of the EU, mainly in Italy, Greece and Spain but also in Bulgaria, France, Hungary, Portugal and Romania and weedy rice is an economically important and widespread weed in rice fields in Europe.

The scope of application EFSA-GMO-UK-2004-04 is for import for food (*e.g.* whole viable grain, processed grains, starch, flour) and feed (*e.g.* hulls, defatted bran, broken rice) uses and processing of LLRICE62, and does not include cultivation. Since the primary use of LLRICE62 is for food and feed, the environmental risk assessment is concerned with indirect exposure through manure and faeces from the gastrointestinal tracts mainly of humans and animals

consuming LLRICE62 and with accidental release into the environment of viable GM grains during transportation and processing.

LLRICE62 has been developed for tolerance to glufosinate by insertion of the *bar* gene that expresses the PAT protein, originating from the soil microorganism, *Streptomyces hygroscopicus* Section 2.2.1).

As this application is not for cultivation, concerns regarding the use of glufosinate on LLRICE62 apply only to imported and processed by-products of LLRICE62 that may have been treated with glufosinate in the countries of origin. However the GMO Panel is aware that glufosinate is used in Europe on other crops and that the risk assessment of this active substance is within the scope of Directive 91/414/EEC concerning the placing of plant protection products on the market.

5.2.1.1 Potential unintended effects on plant fitness due to the genetic modification

The cultivated rice (*Oryza sativa* L.) is an annual self-pollinating crop which has a relatively low percentage of cross-pollination due to the rapid decline in pollen viability with time (OECD, 1999). Seed and pollen dispersal are potential sources of gene flow to conventional rice varieties and to weedy red rice plants. Seeds are the only survival structures.

Field trials to study the agronomic performance traits of LLRICE62 were carried out by the applicant initially in Brazil and Argentina over two growing seasons (1998-1999 and 1999-2000) and were further supplement with data gathered from field trials conducted from 1999 to 2001 in USA in 13 locations in 4 States. Fields were monitored for possible volunteers during one subsequent season. The applicant provided further laboratory and field trials data over 3 years in USA and Brazil on the plant reproductive characteristics of LLRICE62. These data do not show any enhanced fitness or increased invasiveness, and weediness of LLRICE62 plants.

A study by Zhang *et al.* (2003) of deliberate hybridisation between cultivated LLRICE62 plants and weedy red rice plants indicated that potential hybrids have an intermediate fitness between the two parents. In addition, the fields were monitored for possible volunteers during one subsequent season. The monitoring observations have shown no indication of increased persistence or invasiveness of LLRICE62. In addition to the data presented by the applicant, the GMO Panel is not aware of any scientific report of increased fecundity or ferality of herbicide tolerant rice in regions where GM rice is cultivated. There is no information to indicate change in survival capacity, including over-wintering. Also the GMO Panel has noticed from literature reports that the presence of GM and non GM herbicide tolerance traits has not led to an increased invasiveness of volunteers in other crop species such as oilseed rape, maize and soybeans (Saji *et al.*, 2005; Yoshimura *et al.*, 2006; Kim *et al.*, 2006).

If accidental release into the environment occurs, these GM rice plants will only be fitter in the presence of glufosinate which the applicant states is occasionally used in rice growing areas. However since LLRICE62 has no altered survival except when cultivated in the presence of glufosinate, and no altered multiplication or dissemination characteristics, the GMO Panel is of the opinion that, even in case of accidental release into the environment, LLRICE62 is very unlikely to show any enhanced fitness and would behave as conventional rice.

5.2.1.2 Potential for gene transfer

A pre-requisite for any gene transfer is the availability of pathways for the transfer of genetic material, either through horizontal gene transfer of DNA, or vertical gene flow via seed dispersal and cross-pollination.

(a) Plant to bacteria gene transfer

Based on present scientific knowledge and elaborated recently in more detail (EFSA, 2004; EFSA, 2007), gene transfer from GM plants to microorganisms under natural conditions is extremely unlikely, and is more likely to occur primarily through homologous recombination in microorganisms already containing the *bar* gene.

In the case of accidental release and establishment of LLRICE62 in the environment, exposure of microorganisms to transgenic DNA derived from GM rice plants would take place during natural decay of GM plant material and/or pollen in the soil of areas where volunteers might establish.

Food and feed products derived from the GM rice could contain transgenic DNA. Therefore microorganisms in the digestive tract of humans and animals may be exposed to transgenic DNA.

The *bar* gene is known to be a component of soil microbial populations. Taking into account the origin and nature of *bar* gene and the lack of selective pressure in the intestinal tract and/or the environment, the likelihood that horizontal gene transfer of the *bar* gene would confer selective advantages or increased fitness of microorganisms is very limited. For this reason it is very unlikely that the *bar* gene from LLRICE62 would become established in the genome of microorganisms in the environment or human and animal digestive tract. In the very unlikely event that such a horizontal gene transfer would take place, no adverse effects on human and animal health and the environment are expected, as no principally new traits would be introduced or expressed in microbial communities.

(b) Plant to plant gene transfer

The applicant states that most of the LLRICE62 will be grown in the USA and imported into the EU as commodity rice grain for further processing and packaging. A proportion of the imported GM rice will be viable grains in the so called 'paddy rice' fraction. Hybrid grains between LLRICE62 and weedy rice plants might also occur within this fraction.

The applicant has reported that imported paddy rice containing viable grains will be transported in both sealed and bulk containers through some rice growing areas of Europe to processing plants located in these areas. If spillage were to occur along these routes or near the processing plants, viable GM seeds could be dispersed into land cultivating rice and establish populations. However it is unlikely that spillage will result in feral plants establishing around ports, mills, and transit routes as no feral plants currently occur in Europe and there is no indication of changes in fitness or behaviour of this GM rice except in the presence of glufosinate (see Section 5.2.1.1).

The plant to plant gene transfer via cross-pollination from this GM rice is restricted to cultivated conventional rice varieties and weedy rice, as there are no wild or feral *Oryza* spp or other cross compatible plant species in Europe occurring outside of cultivation (OECD, 1999). Rice is largely a self pollinating species and intra-specific cross pollination levels are generally low and decline

rapidly with distance. Considering the intended uses of this LLRICE62 application, the extent of cross-pollination from the GM rice to conventional rice varieties or weedy rice plants in fields will initially depend on the scale of accidental releases into fields, since cross pollination will occur between plants growing in close proximity. Outcrossing would not increase the fitness or invasiveness of volunteers, weedy or wild GM rice except in the presence of glufosinate, which is occasionally used in rice growing areas (see Section 5.2.1.1).

The GMO Panel concludes that there is a possibility that GM rice could enter cultivation through accidental spillage of imported viable GM rice during transport through rice growing areas. The frequency of cross-pollination between the imported viable GM rice and cultivated or weedy rice will be directly related to levels of such spillage and subsequent establishment of GM populations in rice fields.

5.2.1.3 Potential interactions of the GM plant with target organisms

LLRICE62 has been developed for tolerance to glufosinate by insertion of the *bar* gene that expresses the PAT protein, originating from the soil microorganism, *Streptomyces hygroscopicus* Section 2.2.1).

However, considering that the proposed uses of LLRICE62 specifically exclude cultivation, the environmental exposure is mainly limited to accidental release of viable GM grains during transportation and processing, to subsequent establishment of GM populations in rice fields and to possible outcrossing with non GM cultivated or weedy rice plants.

Furthermore there are no specific "target organisms" for LLRICE62 and the introduced trait. Consequently, this was not considered to be an environmental issue by the Member States and by the GMO Panel.

5.2.1.4 Potential interactions of the GM plant with non-target organisms

Considering the proposed uses of LLRICE62 essentially as food, the environmental risk assessment is concerned with indirect exposure through manure and faeces from the gastrointestinal tracts mainly of humans and animals consuming LLRICE62 and with accidental release into the environment of viable GM grains during transportation and processing.

Exposure of soil and water environments to the PAT protein, which is found in nature, from disposal of animal wastes is likely to be very low and localized. Thus exposure of potentially sensitive non-target organisms to the PAT protein is likely to be very low and of no ecological relevance.

Furthermore since LLRICE62 will not be cultivated in Europe, there are no non-target organisms exposed to the GM rice in the field and consequently this was not considered to be an environmental issue by the Member States and by the GMO Panel.

5.2.1.5 Potential interaction with the abiotic environment and biogeochemical cycles

This point was not considered an issue by the Member States or by the GMO Panel. The level of exposure would be so low that potential effects on the abiotic environment and biogeochemical cycles are unlikely.

5.2.2 Monitoring

The objectives of a monitoring plan according to Annex VII of Directive 2001/18/EC are to confirm that any assumption regarding the occurrence and impact of potential adverse effects of the GMO, or its use, in the environmental risk assessment are correct and to identify the occurrence of adverse effects of the GMO, or its use, on human health or the environment which were not anticipated in the environmental risk assessment.

Monitoring is related to risk management, and thus a final adoption of the monitoring plan falls outside the mandate of EFSA. However, the GMO Panel gives its opinion on the scientific quality of the monitoring plan provided by the applicant (EFSA, 2006b). The potential exposure to the environment of LLRICE62 would be through manure and faeces from the gastrointestinal tracts mainly of humans and animals consuming LLRICE62 or through accidental release into the environment of viable GM grains during transportation and processing.

No specific environmental impact of this GM rice was indicated by the environmental risk assessment and thus no case specific monitoring is required.

The general surveillance plan proposed by the applicant includes i) the description of an approach involving operators, reporting to the applicants any observed adverse effect of GMOs on human health and the environment, ii) a coordinating system established by EuropaBio, iii) the use of networks of existing surveillance systems. The applicant will submit a general surveillance report annually and a final report at the end of the consent. In case of confirmed adverse effects, the applicant will immediately inform the European Commission and the Member States.

The GMO Panel is of the opinion that the scope of the monitoring plan provided by the applicant is in line with the intended uses of LLRICE62 since the environmental risk assessment did not cover cultivation and identified no potential adverse environmental effects. The GMO Panel agrees with the reporting intervals proposed by the applicant in the general surveillance plan.

The GMO Panel is of the opinion that appropriate management measures should be in place to prevent LLRICE62 viable grains being spilled into EU rice growing areas (see section 5.2.1.2 (b)). In addition operators should report any spillage of GM rice in rice growing areas and report on the measures taken to remove or destroy any subsequent GM plants. The efficacy of management measures to prevent LLRICE62 spillage into EU rice growing areas and subsequent measures to prevent establishment of GM plants and outcrossing, should be reported as part of the general surveillance activities.

5.3 Conclusion

The scope of application EFSA-GMO-UK-2004-04 is for import for food and feed uses and processing of LLRICE62, and does not include cultivation. Since the primary use of LLRICE62 is for food use, the environmental risk assessment is concerned with indirect exposure through manure and faeces from the gastrointestinal tracts mainly of humans and animals consuming LLRICE62 and with accidental release into the environment of viable GM grains during transportation and processing.

The imported GM rice is likely to include a proportion of viable grains which will be transported through rice growing areas of Europe to processing plants. Accidental release into the

environment is possible and viable GM seeds could be dispersed into land cultivating rice and establish GM populations, which could outcross with non GM cultivated or weedy rice plants. The GMO Panel concludes that there is a possibility that small numbers of GM rice plants could enter cultivation and cross-pollinate with cultivated or weedy rice. However it is unlikely that spillage will result in feral plants establishing around ports, mills, and transit routes as there is no indication of changes in fitness or behaviour of this GM rice, except in the presence of glufosinate.

The GMO Panel advises that appropriate management systems should be in place to restrict seeds of LLRICE62 entering cultivation as the latter requires specific approval under Directive 2001/18/EC or Regulation (EC) No 1829/2003. These should include management measures to prevent LLRICE62 spillage into EU rice growing areas and subsequent establishment of GM plants and outcrossing. The efficacy of these measures should be reported as part of the general surveillance activities. The GMO Panel agrees that the scope of the monitoring plan provided by the applicant is in line with the intended uses of LLRICE62 since the environmental risk assessment excluded cultivation and identified no potential adverse environmental effects. Furthermore the GMO Panel agrees with the reporting intervals proposed by the applicant in the general surveillance plan.

CONCLUSIONS AND RECOMMENDATIONS

The GMO Panel was requested to carry out a scientific risk assessment of the LLRICE62 for food and feed uses, import and processing. The GMO Panel is of the opinion that the molecular characterisation of the DNA insert and flanking regions in LLRICE62 does not raise safety concerns, and sufficient evidence for the stability of the insert structure and of the newly introduced trait was provided. Comparative analysis has shown that LLRICE62 is compositionally and agronomically equivalent to conventional rice, except for the introduced trait. The risk assessment included an analysis of data from analytical studies, bioinformatics, and *in vitro* and *in vivo* studies. The GMO Panel concluded that the LLRICE62 is as safe as its non -GM comparator and that the overall allergenicity of the whole plant is not changed.

The application EFSA-GMO-UK-2004-04 is for food and feed uses, import and processing. There is therefore no requirement for scientific information on possible environmental effects associated with the cultivation of LLRICE62. Considering the scope of the application, not for cultivation, the GMO Panel is of the opinion that unintended environmental effects due to this rice will be no different from that of conventional rice varieties. There is a likelihood of spillage and subsequent spread and establishment of LLRICE62 during transport of paddy rice and the GMO Panel advises that appropriate management systems should be in place to prevent seeds of LLRICE62 entering cultivation. The scope of the monitoring plan provided by the applicant and the reporting intervals are in line with the intended uses of LLRICE62 since cultivation is excluded.

In conclusion, taking into account issues raised by Member States, the GMO Panel considers that, on the basis of the information available for LLRICE62, it is unlikely that LLRICE62 will have any adverse effect on human and animal health or on the environment in the context of its proposed uses.

DOCUMENTATION PROVIDED TO EFSA

1. Letter from the Member State (United Kingdom) dated 18 August 2004, concerning a request for placing on the market of LLRICE62 in accordance with Regulation (EC) 1829/2003.

2. Letter from EFSA to applicant, dated 21 September 2004, with request for additional information to complete the application (Ref. SR/AC/sp (2004) 716).

3. Letter from applicant to EFSA, dated 23 November 2004, providing additional information upon EFSA request

4. Letter from JRC to applicant, dated 20 December 2004 asking additional information.

5. Letter from EFSA to applicant, dated 21 December 2004, with request for a Third Party consultation (Ref. SR/DD/KL (2004) 1132).

6. Letter from EFSA to applicant, dated 14 January 2005, delivering the 'Statement of Validity' for application EFSA-GMO-UK-2004-04, LLRICE62 submitted by Bayer CropScience under Regulation (EC) 1829/2003 (Ref. SR/AC/jq (2005) 067).

7. Letter from applicant to EFSA, dated 24 January 2005, in reply to EFSA letter dated 21 December 2004 - Third Party consultation.

8. Letter from applicant, dated 27 January 2005, providing EFSA with an updated version of the application EFSA-GMO-UK-2004-04 submitted by Bayer CropScience under Regulation (EC) 1829/2003:

   Part I – Technical dossier

   Part II – Summary

   Part III – Cartagena Protocol

   Part IV – Labelling and Unique Identifier

   Part V – Samples and Detection

   Part VI – Additional information for GMOs

9. Letter from EFSA to applicant, dated 21 March 2005, with request for additional information (Ref. SR /AC/jq (2005) 327).

10. Letter from JRC to EFSA, dated 31 March 2005, with complete application (Ref. JRC I06-BGMO/GVDE/SC/D (2005) (79)7449.

11. Letter from applicant to EFSA, dated 10 May 2005, providing additional information upon EFSA request.

12. Letter from EFSA to applicant, dated 12 July 2005, with request for additional information (Ref. SR /AC/jq (2005) 918).

13. Letter from EFSA to applicant, dated 27 March 2006, with request for Public access CDs.

14. Letter from applicant to EFSA, dated 26 April 2006, providing the Public access version requested by EFSA.

15. Letter from EFSA to applicant, dated 19 September 2006, with request for a Third Party consultation.

16. Letter from applicant to EFSA, dated 29 March 2007, providing additional information upon EFSA request.

17. Letter from EFSA to applicant, dated 5 June 2007, with request for additional information (Ref. SR/AC/shv (2007) 2176776).

18. Letter from applicant to EFSA, dated 7 June 2007, providing additional information upon EFSA request.

19. Letter from EFSA to applicant, dated 18 June 2007, with request for additional information (Ref. SR/AC/shv (2007) 2203660).

20. Letter from applicant to EFSA, dated 4 July 2007, providing additional information upon EFSA request.

21. Letter from EFSA to applicant, dated 21 September 2007, with request for additional information (Ref. SR/AC/shv (2007) 2396535).

22. Letter from applicant to EFSA, dated 11 October 2007, providing additional information upon EFSA request.

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