

Health risks: Bayer's Genetically Engineered Liberty Link (LL) RICE 601

Summary of findings

The genetically engineered (GE) herbicide tolerant rice, LLRICE601, is unapproved for cultivation and human consumption. It is not approved in any country worldwide. LLRICE601 should not be approved for human consumption by the United States Department of Agriculture Animal and Plant Health Inspection Service (USDA APHIS) not least because the data submitted to APHIS by Bayer is woefully inadequate. The rice contains additional and unwanted gene sequences, derived from the imprecise method used to manipulate its genome. This means there could be compounds in the rice that indeed might be problematic for human health. Since the company did not provide comprehensive data there are good reasons for concern. The food safety of LLRICE601 is unknown.

Risks should not be taken with public health or the environment for the convenience of Bayer or of any government. Greenpeace demands that APHIS reject the Bayer application and demands that the European member states secure that no products contaminated with the unapproved rice variety are sold.

Background

On 18th August 2006, the US Department of Agriculture announced that Bayer CropScience had reported that rice from the 2005 crop being sold commercially in the USA had been found to be contaminated with a GE variety, LLRICE601, which is not approved for growing or consumption anywhere in the world. The GE rice, developed by Bayer CropScience, is genetically engineered to be tolerant to the herbicide glufosinate (trade name: Liberty or Basta), but development of the LLRICE601 variety ended in 2001. Two other varieties of glufosinate tolerant rice, also developed by Bayer, LLRICE62 and LLRICE06, are approved in the USA but are not being grown commercially.¹

Subsequent to the announcement, US rice shipments to the EU were found to be contaminated.² Japan suspended imports of long-grain rice from the USA and the European Commission announced that imports of rice would only be allowed if they were accompanied by a certificate demonstrating they did not contain the unauthorised GE rice.

The LLRICE601 variety is not approved (or "deregulated" in the US), and no trace of contamination is allowable by law in the EU. Bayer has now submitted a dossier to the US authorities and requested approval (or petitioned for deregulation) of LLRICE601 in the US. Presumably Bayer hope that deregulation in the US will legalise the contamination in the US and aid a positive safety assessment in the EU. A positive safety assessment in the EU would mean a tolerance for the unapproved rice of up to 0.5 %.

The basis of Bayer's application for US approval is that the unauthorised line, LLRICE601, is similar to the two lines of GE glufosinate-resistant varieties of rice that are already authorised in the US, namely LLRICE62 and LLRICE06. However, line LLRICE601 is not similar to the two approved lines, except that the desired trait is the same. LLRICE601 was produced by a

¹ <http://www.gmcontaminationregister.org/>

² <http://www.greenpeace.org/international/news/banned-290906>

completely different method of genetic engineering. In addition, the dossier submitted by Bayer is wholly inadequate and does not contain enough information for any regulatory authority to begin to assess this GE rice. This document details the gaps in the data and concludes that this GE rice should not be given deregulated status in the USA and that the EU should continue to reject shipments containing even trace amounts of this GE rice, as the food safety of this rice is completely unknown and untested.

1) LLRICE601 is not similar to other GE glufosinate tolerant rice lines

Bayer's application to the Animal and Plant Health Inspection Service (APHIS) of the US Department of Agriculture (USDA) is entitled "Extension of the Determination of Nonregulated Status for Glufosinate-Tolerant Rice (98-329-01p)^{3,4} (hereafter called "Bayer's Dossier"). In this document Bayer is seeking to include authorisation for LLRICE601 in with the existing authorisations (technically deregulation) for LLRICE62 and LLRICE06. APHIS published a draft environmental assessment in early September⁵ in support of this application (hereafter called the APHIS assessment).

Table 1. Fundamental differences between LLRICE601 and the two other GE rice lines (LLRICE62 and LLRICE06) showing fundamental differences

	LLRICE62	LLRICE06	LLRICE601
Method of transformation⁶	direct gene transfer using gene gun	direct gene transfer using gene gun	<i>Agrobacterium</i> -mediated transformation
Rice variety used for transformation	medium grain variety Bengal	medium grain variety M202	long grain variety Cocodrie
promoter	35S CaMV promoter		slightly longer 35S CaMV promoter; 2 nd copy of the promoter
terminator	35S CaMV terminator		<i>nos</i> (nopaline synthase) terminator (truncated)
PAT protein			Identical to that in LL cotton ⁷
level of PAT protein expressed	High	Low	Lower than LLRICE62 and LLRICE06 in seeds BUT higher than LLRICE06 in leaf ⁸

The APHIS assessment states their preliminary decision to make LLRICE601 non-regulated on the basis that it is similar to the other GE LL rice strains. However, this is not so. There are

³ Bayer Crop Science petition number 06_23401p - Application for an extension of the determination of nonregulated status for glufosinate-tolerant rice (98-329-01p): transformation event LLRICE601. OECD unique identifier BCS-OS003-7. See www.aphis.usda.gov/brs/not_reg.html

⁴ In the US 'Non-Regulated Status' allows a GMO to be grown without the use of a field trial permit. It is similar to a marketing consent for growing in the EU under EU Directive 2001/18/EC.

⁵ USDA/APHIS Draft Environmental Assessment. In response to Bayer CropScience Petition 06-234-01P seeking extension of determination of non-regulated status for glufosinate resistant rice, *Oryza sativa*, event LLRICE601. http://www.aphis.usda.gov/brs/aphisdocs/06_23401p_pea.pdf and announced in the Federal Register 8th September 2006. http://www.aphis.usda.gov/brs/fedregister/BRS_20060908a.pdf

⁶ USDA/APHIS draft EA 06-234-01P, page 18

⁷ "The sequence of the PAT protein produced in LLRICE601 is identical to the sequence produced in the approved cotton line LL Cotton25. These sequences vary from the LLRICE06 and -62 PAT proteins by a single amino acid at position 2 where the former have an aspartic acid residue and the latter have a serine.", USDA/APHIS draft EA 06-234-01P, page 19

⁸ "In the seed, the level of PAT protein in LLRICE601 is below the level in -06 and -62. In the leaf tissues, the level of PAT protein in -601 is much less than the level in -62 but is slightly higher than the level in -06."

fundamental differences in the production of LLRICE601 (see Table 1) that means it should be assessed separately from the other lines. Importantly, LLRICE601 was produced using *Agrobacterium tumefaciens*, whereas LLRICE62 and LLRICE06 are produced by the gene gun method (or direct gene transfer). LLRICE601 contains a different terminator (“stop” code) than LLRICE62 and LLRICE06.

Hence, LLRICE601 is not very similar at all to LLRICE62 or LLRICE06. For the event LLRICE601 a different rice-variety was transformed with different gene constructs using different transformation methods. In fact, the only similarities between the GE rice events LLRICE62, LLRICE06 and LLRICE601 are that all three were produced by Bayer⁹ and that all three GE rice events are resistant to the herbicide glufosinate (Liberty). Therefore, the data used to prove the food and environmental “safety” of LLRICE62 and LLRICE06 cannot, in any way, be used to aid the application for approval of LLRICE601.

2) GE rice LLRICE601 contains additional and unwanted gene sequences

In the Bayer dossier, most of the important molecular data describing the sequences actually inserted into the DNA and their expression is covered by commercial confidentiality claims. It is therefore impossible for Greenpeace to comment on these sections. It is clear, however, the GE rice LLRICE601 contains additional and unwanted gene sequences. These are unintentionally created by the crude and old-fashioned technology that is genetic engineering. This means there could be compounds in the rice that might be problematic for human health.

The LLRICE601 contains one functioning gene insert containing the 35S CaMV promoter, the bar gene (the active gene) and a truncated “nos” terminator (the stop signal). Additionally, the LLRICE601 seems to contain a second copy of all or part of the 35S CaMV promoter (the “on” switch for the inserted genes) at an unknown, random location in the genome. It seems likely that the implications of this second insert have never been examined. The 35S CaMV promoter is what is known as a “constitutive” promoter, that is, it acts as a constant “on” switch for an unknown number of genes, in all cells. Indeed, there have been concerns about the use of this particular promoter in GE foodstuffs¹⁰ because it may unintentionally turn genes “on” even in mammals.

*“Details on molecular characterization of LLRICE601 were submitted to APHIS in a report, Molecular Characterization of Glufosinate-Tolerant rice transformation event LLRICE601, dated August 4, 2006. Bayer included a Southern blot analysis of DNA from lines containing the event LLRICE601 consistent with the conclusion that only a single bar gene flanked by an intact 35S promoter and a truncated nos terminator were inserted into the genome. In addition, the Southern blot revealed extra bands for the 35S CaMV promoter suggesting a second copy of the promoter inserted elsewhere in the genome. The random insertion of an extra 35S promoter fragment in the rice genome is unlikely to have any consequence as the effectiveness of the promoter is dependent on it inserting close enough to DNA encoding a functional gene. At a low frequency, **it could potentially insert near enough to another gene to alter the expression of a native rice gene. Alternatively, it could integrate within a native rice gene and disrupt its function.**”*
[APHIS assessment, page 19]

In GE LLRICE601, the unintended, additional 35S CaMV promoter “on” switch may interfere with the normal expression of native rice genes. It could cause genes that should be inactive to become active. The consequences of this are not known, but require a full and thorough investigation before any conclusion regarding their safety equivalence to non-GE rice can be made.

⁹ “AgrEvo, whose parent company Hoechst AG merged with Rhone-Poulenc to form Aventis in 1999. Aventis was acquired by Bayer in 2002.”, USDA/APHIS draft EA 06-234-01P, page 16

¹⁰ Myhre, M.R., Fenton, K.A., Eggert, J., Nielsen, K.M. & Traavik, T. 2006. The 35S CaMV plant virus promoter is active in human enterocyte-like cells. *European Food Research Technology* 222: 185–193.

3) Essential data on composition and health studies are non-existent

In Bayer's dossier, they provide only summary compositional data, not the full data normally supplied by companies. The additional 35S promoter inserted at random at an unknown location with the genome of LLRICE601 make the compositional analysis of LLRICE601 highly important. The compositional analysis might reveal significant differences that indicate there are differences between the GE and non-GE sister line that could have been caused by the additional promoter. Any such differences would be important for food safety, and could indicate adverse effects. Therefore, there are lots of unanswered questions concerning transparency and safety.

The data supplied in Bayer's dossier would not be complete enough for a submission to the EU. In the EU, food safety is regulated by the European Food Safety Authority (EFSA). EFSA have laid out their requirements for data in a guidance document for companies such as Bayer.¹¹ Table 2 makes it clear that many of the data that would be required for an assessment of a GE plant by EFSA in the EU are missing.

Table 2 Comparison of data requirements for EU application for approval of a GE plant and that actually contained in Bayer's submission to US authorities	
Data requested by European Food Safety Authority (EFSA)¹²	Included in Bayer's petition for non regulated status?
General Information	Yes
Information related to rice	Yes
Information on the genetic modification	
Method of modification	Yes
Used vector	Yes
Complete sequence of the DNA used	No
Information GM Plant	
General information on the GM Plant	Yes
Information on inserted sequences	
Expression of the inserted sequences	No
Comparable to parental plant	No
Stability	Not known*
Ability to transfer DNA	Yes
Information on toxic or allergic effects	
Comparable parental plant grown under same conditions	No
Field trials with sufficient statistical power	No
Key macro- and micro-nutrients, anti-nutritional compounds, and natural toxins should be determined ¹³	No
Risk assessment on usage of gm plant in processed food or feed	Not known*

¹¹ EFSA Guidance document of the scientific panel on genetically modified organisms for the risk assessment of genetically modified plants and derived food and feed. http://www.efsa.europa.eu/en/science/gmo/gmo_guidance.html
Hereafter called "EFSA guidance"

¹² EFSA Guidance document of the scientific panel on genetically modified organisms for the risk assessment of genetically modified plants and derived food and feed. http://www.efsa.europa.eu/en/science/gmo/gmo_guidance.html
Hereafter called "EFSA guidance"

¹³ EFSA Guidance, page 25: "Similarly, anti-nutritional compounds, such as digestive enzyme inhibitors, and important identified allergens should be studied. Compounds other than the key nutrients, key toxins, and important anti-nutrients and allergens identified by the OECD consensus documents may be included in the analyses on a case-by-case basis. The OECD consensus documents, therefore, provide a minimum list of compounds for analysis."

Table 2 Comparison of data requirements for EU application for approval of a GE plant and that actually contained in Bayer's submission to US authorities	
Data requested by European Food Safety Authority (EFSA)¹²	Included in Bayer's petition for non regulated status?
Concentration of PAT protein	Not known*
Consequences on all genetic modification (e.g. Second 35 S promoter) ¹⁴	No
Toxicological studies which are carried out should be conducted using internationally agreed protocols.	Not known*
Animal test with rodents ¹⁵ [only recommended, if no data already published]	No
Intensive animal tests with rodents if any unexpected effects ¹⁶	No
Growth studies with broiler chicken ¹⁷	No
Quantities of known rice allergenic proteins ¹⁸	No
Data on allergy in workers or in farmers ¹⁹	No
Nutritional assessment of GM food	No

* Not known. This information is not given in the dossier but could be declared CBI (Confidential Business Information)

There are internationally agreed lists of which compositional data should be submitted in applications for market approval of GE crops. Regulatory authorities, such as EFSA in the EU, make use of these lists to determine which compounds are important to analyse for a particular plant when considering the food safety of a GE plant. The Organisation for Economic Co-operation and Development (OECD) has produced what is termed a "consensus document" for rice that lists the type of data that should be included for the compositional analysis of GE rice in submissions

for market approval.²⁰ This document lists the anti-nutritional compounds in rice as: phytin; allergens such as glyoxalase; trypsin Inhibitor; oryzacystatin; alpha-amylase subtilisin inhibitor; and lectins. None of these anti-nutrients have been analysed in the safety analysis of LLRICE601.

¹⁴ EFSA Guidance, page 27: "Thus, the toxicological testing would not only include studies on newly expressed proteins but also the consequences of any genetic modification (e.g. gene silencing or over-expression of an endogenous gene). In principle, the safety assessment must consider the presence of new proteins expressed as result of the genetic modification, the potential presence of other new constituents and/or possible changes in the level of natural constituents beyond normal variation. These potential deviations from the conventional counterparts may require different toxicological approaches and varying degrees of testing."

¹⁵ EFSA Guidance, page 28: "Normally a 28-day oral toxicity study with the newly expressed protein in rodents should be performed according to OECD guideline 407 (OECD, 1995)."

¹⁶ EFSA Guidance, page 29: "If the composition of the GM plant is modified substantially, or if there are any indications for the potential occurrence of unintended effects, based on the preceding molecular, compositional or phenotypic analysis, not only new constituents, but also the whole GM food/feed should be tested. In such a case, the testing programme should include at least a 90-day toxicity study in rodents."

¹⁷ EFSA Guidance, page 29: "Supplemental information on the possible occurrence of unintended effects may be obtained from comparative growth studies conducted with young rapidly growing animal species (broiler chicks as animal model for non-ruminants; lambs for ruminants; or other rapidly growing species). Because of their rapid weight gain such animals are sensitive to the presence of certain undesirable substances in their feed. Studies of this type are, however, limited to those materials suitable for inclusion in their diets and which can be nutritionally matched to a suitable control diet."

¹⁸ EFSA Guidance, page 32: "If the host of the introduced gene is known to be allergenic, any potential change in the allergenicity of the whole GM food should be tested by comparison of the allergen repertoire with that of the conventional non-GM variety."

¹⁹ EFSA Guidance, page 33: "In addition, data on the prevalence of occupational allergy in workers or in farmers who have significant exposure to GM plant and crops, or to the airborne allergens they may contain, will provide useful information for the risk assessment process."

²⁰ Organisation for Economic Co-operation and Development (OECD) 2004.. Consensus Document on Compositional Considerations for New Varieties of Rice (*Oryza Sativa*) Key Food and Feed Nutrients and Anti-Nutrients. Series on the Safety of Novel Foods and Feeds, No 10
http://www.oecd.org/document/9/0,2340,en_2649_34385_1812041_1_1_1_1,00.html

These data should have been submitted and would have been considered an integral part of the assessment by EFSA.

In Europe, EFSA has been criticised for being pro-GE and always giving a positive opinion of GE crops regardless of the data. However, for the first time, EFSA have admitted uncertainties with the food safety of the GE rice LLRICE601. Their conclusions are important as EFSA would have access to the CBI. EFSA state²¹ that:

“Although extensive data have been presented regarding the molecular characterisation of the intended insert, no full molecular characterization was presented and only limited summary data have been provided regarding the compositional analysis of the rice and agronomic and nutritional equivalence to conventional rice. It is therefore not possible to conclude on the safety of LLRICE601 itself, in accordance with the EFSA guidance for risk assessment.”

The EFSA further pick out the following specific concerns:

- that there is a lack of analysis on a second copy of the CaMV 35S promoter, which has been inserted at a separate location to the herbicide tolerance gene and its promoter and termination regions.
- that Bayer have not provided the raw data with which to assess the morphology, agronomic performance and compositional analysis of LLRICE601.

Difference in Grain yield

What little agronomic data Bayer have supplied in their dossier indicates that there could be differences in grain yield, with lower yields from the LLRICE601 rice compared to a similar non GE line. It is tempting to speculate that this may be why Bayer abandoned development of this line. Further studies on yield, at least, are required.

No wholesome tests

Normally, the results of feeding trials are submitted in support of the application for approval of a GE crop. These feeding trials are important as often they can reveal significant differences, even though they may only be short term studies. Animal feeding studies have not been performed with LLRICE601 – there is simply no data available.

There are enormous gaps in the data submitted by Bayer in support of the request for approval of LLRICE601. Given the molecular irregularities (with the extra CaMV 35S promoter inserted at random, at an unknown location within the genome), these data gaps become important. Although the assessment of GE crops is usually inadequate as no assessment can cope with the potential for unexpected and unpredictable effects that GE crops are prone to, it is clear that Bayer's dossier and APHIS' assessment do not provide any reassurance of the food safety of LLRICE601. Indeed, even EFSA, much criticised for its pro-GE stance has concluded that there is not enough data to form an assessment.

Therefore;

- **Greenpeace maintains that the food safety of LLRICE601 is completely unknown;**
- **Greenpeace demands that the petition for GE rice LLRICE601 be refused and that Europe continues to keep contaminated rice out of the EU.**

²¹ Statement of the Scientific Panel on Genetically Modified Organisms in response to the request of the European Commission on inadvertent presence of genetically modified rice LLRICE601 adopted on 14 September 2006. http://www.efsa.europa.eu/en/science/gmo/statements0/efsa_statement_gmo_LLRI601.html