FOOD STANDARDS
Australia New Zealand
Te Mana Kounga Kai – Ahitereiria me Aotearoa

19 March 2008
[4-08]

FINAL ASSESSMENT REPORT

APPLICATION A589

FOOD DERIVED FROM GLUFOSINATE AMMONIUM-TOLERANT RICE LLRICE62

For Information on matters relating to this Assessment Report or the assessment process generally, please refer to http://www.foodstandards.gov.au/standardsdevelopment/

Executive Summary

An Application has been received from Bayer CropScience Pty Ltd seeking to amend the *Australia New Zealand Food Standards Code* (the Code) to approve food derived from a genetically modified (GM) variety of rice, LLRICE 62, under Standard 1.5.2 – Food produced using Gene Technology. This Standard requires that GM foods undergo a pre-market safety assessment before they may be sold in Australia and New Zealand.

LLRICE 62 is tolerant to the herbicide glufosinate ammonium through the addition of a bacterial gene (*bar*). Expression of the *bar* gene produces an enzyme, phosphinothricin acetyltransferase (PAT) which inactivates phosphinothricin (PPT), the active constituent of glufosinate ammonium herbicides, allowing the crop to grow in the presence of the herbicide. No marker genes are present in LLRICE62.

Rice line LLRICE 62 is intended to be grown overseas, principally in rice growing regions of the United States. Once the grain is commercialised however, rice products imported to Australia and New Zealand could contain derivatives of LLRICE 62. Approval is therefore necessary before these products could enter the Australian and New Zealand markets. LLRICE62 is not intended for cultivation in either Australia or New Zealand and, to date, no environmental approvals have been sought.

Safety assessment

FSANZ has completed a comprehensive safety assessment of food derived from glufosinate ammonium-tolerant rice LLRICE62, as required under Standard 1.5.2. The assessment included consideration of: (i) the genetic modification to the plant; (ii) the potential toxicity and allergenicity of the novel protein; and (iii) the composition of LLRICE62 compared with that of conventional rice varieties.

The assessment of this Application identified no public health and safety concerns. On the basis of the available evidence, including detailed studies provided by the Applicant, food derived from glufosinate ammonium-tolerant rice LLRICE62 is considered as safe and wholesome as food derived from other commercial rice varieties.

Labelling

Food derived from glufosinate ammonium-tolerant rice LLRICE62 will be required to be labelled as genetically modified if novel DNA and/or novel protein is present in the final food. Studies conducted by the Applicant show that the novel protein is present at low levels in the rice grain. Some processed derivatives such as rice bran oil would be unlikely to contain detectable plant DNA or proteins and would not require labelling.

Labelling addresses the requirement of section 18(1)(b) of the *Food Standards Australia New Zealand Act 1991* (FSANZ Act): the provision of adequate information relating to food to enable consumers to make informed choices.

Impact of regulatory options

Two regulatory options were considered in the assessment: (1) no approval; or (2) approval of food derived from glufosinate ammonium-tolerant rice LLRICE62 based on the conclusions of the safety assessment.

Following analysis of the potential costs and benefits of each option on affected parties (consumers, the food industry and government), approval of this application is the preferred option as the potential benefits outweigh the costs associated with the approval, in comparison with not approving LLRICE62.

Purpose

The Applicant seeks amendment to Standard 1.5.2, to include food derived from glufosinate ammonium-tolerant rice LLRICE62 in the Table to clause 2.

Decision

Amend Standard 1.5.2 – Food produced using Gene Technology, to include food derived from glufosinate ammonium-tolerant rice LLRICE62 in the Table to clause 2.

Reasons for Decision

An amendment to the Code approving food derived from glufosinate ammonium-tolerant rice LLRICE62 in Australia and New Zealand is approved on the basis of the available scientific evidence, for the following reasons:

- the safety assessment did not identify any public health and safety concerns associated with the genetic modification used to produce glufosinate ammonium-tolerant rice LLRICE62;

- food derived from glufosinate ammonium-tolerant rice LLRICE62 is equivalent to food from the conventional counterpart and other commercially available rice varieties in terms of its safety for human consumption and nutritional adequacy;

- labelling of certain food commodities derived from glufosinate ammonium-tolerant rice LLRICE62 will be required if novel DNA and/or protein is present in the final food; and

- a regulation impact assessment process has been undertaken that also fulfils the requirement in New Zealand for an assessment of compliance costs. The assessment concluded that the preferred option is option 2, an amendment to the Code.

Consultation

The Initial and Draft Assessments were each open for public comment for a period of six weeks. Eight submissions were received during the first consultation period and nineteen submissions were received during the second round. A summary of all submissions is attached to this Report (Attachment 3).

FSANZ has taken the submitters' comments into account in preparing the final assessment of this application. Specific issues relating to glufosinate ammonium-tolerant rice LLRICE62 have been addressed in the Report.

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INTRODUCTION

An Application was received from Bayer CropScience Pty Ltd on 6 September 2006 seeking approval in the Code for food derived from glufosinate ammonium-tolerant rice, known as LLRICE62, under Standard 1.5.2 – Food produced using Gene Technology.

A Final Assessment of the Application has been completed, including a comprehensive safety assessment and consideration of issues raised in two rounds of public consultation.

1. Background

LLRICE62 is a genetically modified (GM) variety of rice that is tolerant to the herbicide glufosinate ammonium by the addition of a bacterial gene, known as *bar*, to the rice genome. This gene encodes the enzyme phosphinothricin acetyltransferase (PAT), which inactivates the herbicide. The purpose of the modification is to provide growers with a line of rice that more effectively allows for weed control without affecting the crop.

LLRICE62 has been developed primarily for cultivation in overseas countries where the herbicide will be registered for use on tolerant crops. It has already been approved for food use in the United States of America (2000), Canada (2006), Argentina (2006) and the Russian Federation (2003).

1.1 Previous consideration

The public health and safety issues associated with the use of the *bar* gene from *Streptomyces hygroscopicus* for conferring tolerance to glufosinate ammonium herbicides in GM plants have been considered by FSANZ on previous occasions. Numerous glufosinate ammonium-tolerant varieties of cotton, canola and soybean, containing the *bar* gene, are approved under Standard 1.5.2 (see Applications A372, A375, A380, A381, A386, A446, A481, A518, A533, A543).

2. The Issue / Problem

Standard 1.5.2 requires that a GM food undergo a pre-market safety assessment before it may be sold in Australia and New Zealand. Foods that have been assessed under the Standard, if approved, are listed in the Table to clause 2 of the Standard.

The Applicant has developed LLRICE62, a variety of GM rice tolerant to the herbicide glufosinate ammonium. Although commercial release of the grain and cultivation will be in overseas countries, it would be possible for imported rice products to include LLRICE62. The Applicant is therefore seeking an amendment to Standard 1.5.2 to approve food derived from LLRICE62 in Australian and New Zealand markets.

Food derived from LLRICE62 must be assessed for safety before it can be permitted for food use in Australia and New Zealand. An amendment to the Code must be approved by the FSANZ Board, and subsequently be notified to the Australia and New Zealand Food Regulation Ministerial Council (Ministerial Council). An amendment to the Code may only be gazetted once the Ministerial Council process has been finalised.

3. Objectives

The objective of this assessment is to determine whether it would be appropriate to amend the Code to approve the use of food derived from LLRICE62 under Standard 1.5.2. In developing or varying a food standard, FSANZ is required by its legislation to meet three primary objectives, which are set out in section 18 of the FSANZ Act. These are:

- the protection of public health and safety;
- the provision of adequate information relating to food to enable consumers to make informed choices; and
- the prevention of misleading or deceptive conduct.

In developing and varying standards, FSANZ must also have regard to:

- the need for standards to be based on risk analysis using the best available scientific evidence;
- the promotion of consistency between domestic and international food standards;
- the desirability of an efficient and internationally competitive food industry;
- the promotion of fair trading in food; and
- any written policy guidelines formulated by the Ministerial Council.

4. Key Assessment Questions

The Initial Assessment of this Application identified the key question: Is food derived from LLRICE62 rice as safe for human consumption as food from conventionally produced rice? In addressing this question, FSANZ has considered information provided by the Applicant specifically relating to LLRICE62, previously held information relating to the safety of the novel protein, PAT, when present in food, resource material including published scientific literature and general technical information available in the public domain. The summary and conclusions from the full Safety Assessment Report (at **Attachment 2**) are presented below.

RISK ASSESSMENT

5. Risk Assessment Summary

Rice is a staple food for about half of the world's population and has a long history of use as a nutritious crop for animal feed. Many different cultivars of the predominant species, *Oryza sativa*, have been developed for diverse agricultural conditions. The morphology, physiology, agronomy, genetics and biochemistry of this species have been intensively studied over a long period.

Glufosinate ammonium (or phosphinothricin, L-PPT) is a non-selective, contact herbicide that provides post-emergence control of many broadleaf and grassy weeds.

The mode of action of the herbicide is to inhibit the activity of glutamine synthetase, an essential enzyme involved with nitrogen metabolism in plants. The inhibition of glutamine synthetase results in an over accumulation of ammonia in cells, which typically leads to plant death. In LLRICE62, the *bar* gene from *Streptomyces hygroscopicus* has been inserted into the rice genome. This gene expresses the enzyme phosphinothricin acetyltransferase (PAT) which chemically inactivates the herbicide. The production of PAT by LLRICE62 enables the post-emergence use of glufosinate ammonium herbicides without adverse effects to the crop.

In conducting a safety assessment of food derived from LLRICE62, a number of criteria have been addressed including: a characterisation of the transferred gene, its function and stability in the rice genome; the changes to the rice at the level of the DNA and protein particularly in the edible portions of the plant; detailed compositional analyses; and the potential for the newly introduced protein to be either allergenic or toxic in humans.

5.1 Description of the Genetic Modification

The molecular characterisation analyses on LLRICE62 rice confirm the presence of one intact functional copy of the *bar* gene expression cassette, inserted at a single locus in the rice genome. Fragments corresponding to partial genes, regulatory elements or additional vector backbone sequences were not detected. The precise boundaries of the inserted DNA in LLRICE62 have been fully characterised, and no changes to the sequence were introduced during the transformation process. No marker genes encoding antibiotic resistance are present in LLRICE62.

A complete sequence of the *Oryza sativa* genome has been published. Bioinformatics studies showed that the site of integration of novel DNA in LLRICE62 is on chromosome 6. Further sequence analysis indicated that the insertion site in LLRICE62 is in a region of repeat elements which make up more than 35% of rice genomic DNA.

5.2 Characterisation of Novel Protein

LLRICE62 is tolerant to glufosinate ammonium through the expression in the plant of the bacterial enzyme PAT. This enzyme chemically converts the herbicide to the metabolite N-acetyl-L-PPT, which is unable to bind to the plant glutamine synthetase.

The PAT protein is expressed in LLRICE62 at very low levels in the unprocessed grain. When grown under normal field conditions including treatment with glufosinate ammonium, PAT constitutes 12.1 µg/g fresh weight in grain which corresponds to about 0.02% of the crude protein. PAT was detected at low levels in all processed commodity fractions derived from the grain, with the exception of rice bran oil which contains no plant proteins.

The potential toxicity and allergenicity of the PAT protein have been assessed previously by FSANZ and no safety concerns have been identified. No adverse effects were identified in acute toxicity studies in mice using purified PAT protein. The PAT protein does not exhibit sequence similarity with known protein toxins or allergens, and is degraded in conditions that mimic human digestion. Based on bioinformatic, biochemical and acute toxicity studies, PAT is considered non-toxic in humans and is unlikely to be allergenic.

Reviews of the safety of the metabolites resulting from the inactivation of glufosinate ammonium by PAT concluded that the metabolites are less toxic or equivalent in toxicity to the parent compound in humans.

5.3 Compositional Analyses

Compositional studies were conducted over different seasons and environments to establish the nutritional adequacy of LLRICE62 and compare it with the conventional parental line and other commercial rice varieties under typical cultivation conditions. The constituents measured were proximates (crude protein, fat, ash, fibre and moisture), amino acids, fatty acids, vitamins, minerals, and a small number of anti-nutrients relevant to rice grain.

No differences of biological significance were found between LLRICE62 and the conventional counterpart variety. Small differences in some nutrients were noted however the changes were not consistent across trial sites and do not indicate an overall pattern of change that could be attributed to the genetic modification. Based on the high degree of similarity in composition between LLRICE62 and conventionally produced rice varieties, no food safety issues were identified.

5.4 Nutritional Impact

The detailed compositional studies are considered adequate to establish the nutritional adequacy of food derived from LLRICE62 rice. Results from two feeding studies, one in growing-finishing swine and the other in broiler chickens, further demonstrate that LLRICE62 is nutritionally equivalent to its conventional counterpart. Animals fed diets containing LLRICE62 were able to grow normally and produce food products with qualities and characteristics typical of animals fed on conventional diets. The introduction of products derived from LLRICE62 into the food supply is therefore expected to have minimal nutritional impact.

5.5 Conclusion

No potential public health and safety concerns have been identified in the comprehensive assessment of glufosinate ammonium-tolerant rice LLRICE62. On the basis of the data provided in the Application, and other available information, food derived from LLRICE62 is considered as safe and wholesome as food derived from the conventional varieties of rice.

RISK MANAGEMENT

6. Options

There are no non-regulatory options that may be considered in response to this Application. The two regulatory options available for this Application are:

6.1 Option 1 – Not approve food derived from LLRICE62

Maintain the *status quo* by not amending Standard 1.5.2 to approve food derived from glufosinate ammonium-tolerant rice line LLRICE 62.

6.2 Option 2 – Approve food derived from LLRICE62

Amend Standard 1.5.2 to permit the sale and use of food derived from glufosinate ammonium-tolerant rice LLRICE 62, with or without specified conditions in the Table to clause 2 of the Standard.

7. Impact Analysis

FSANZ is required to consider the impact of various regulatory (and non-regulatory) options on all sectors of the community in Australia and New Zealand.

7.1 Affected Parties

The affected parties could include the following:

- Consumers of rice and rice products;

- Food industry sectors such as:

    - Importers of wholesale food ingredients;
    - Importers of processed rice products;
    - Processors and manufacturers;
    - Retailers; and

- Government generally, where a regulatory decision may impact on trade or WTO obligations, and enforcement agencies in particular who will need to ensure that any approved products are correctly labelled.

The cultivation of rice line LLRICE62 in Australia or New Zealand could have an impact on the environment, which would need to be assessed by the Office of the Gene Technology Regulator (OGTR) in Australia, and by various New Zealand Government agencies including the Environmental Risk Management Authority (ERMA) and the Ministry of Agriculture and Fisheries (MAF) before growing in either country could be permitted. LLRICE62 has been developed primarily for agricultural production overseas and, at this stage, the Applicant has no plans for cultivation in either Australia or New Zealand.

7.2 Benefit Cost Analysis

In the course of developing food regulatory measures suitable for adoption in Australia and New Zealand, FSANZ is required to consider the impact of all options on the community, including consumers, the food industry and governments in both countries. The regulatory impact assessment identifies and evaluates, though is not limited to, the anticipated costs and benefits of the regulation, and its health, economic and social impacts.

In preparing this benefit cost analysis, FSANZ has assumed that LLRICE62 would eventually be a commercialised variety of rice available for agricultural production in certain countries. In these circumstances, food derived from LLRICE62 would be available for sale where appropriate regulatory approvals have been obtained. If LLRICE62 is not released commercially, GM rice would not be widespread in the market and the impact analysis (below) would not apply.

7.2.1 Option 1 – not approve food derived from LLRICE62

Consumers: Possible restriction in the availability of rice products if LLRICE62 is eventually commercialised and used in foods intended for import into Australia and New Zealand. No impact on consumers wishing to avoid GM foods, as food from LLRICE62 rice is not currently permitted in the food supply.

Industry: Possible restriction on importing rice and rice products from countries where LLRICE62 is approved and grown (after commercialisation), due to commingling practices, which could increase the cost of imports. The range of products offered for sale by food retailers could also be restricted.

Potential longer-term impact – any successful WTO challenge has the potential to impact adversely on the food industry. Possible costs to Australian exporters if high-value markets demanded certification on GM status of rice products (although these costs would be expected regardless of regulatory status in Australia and New Zealand if LLRICE62 is commercialised in other countries).

Government: Potential impact if considered inconsistent with WTO obligations but impact would be in terms of trade policy rather than in government revenue. Potential costs include resources required for monitoring and ensuring compliance with the Code.

7.2.2 Option 2 – approve food derived from LLRICE62

Consumers: Access to a wider range of imported rice products at lower prices as there would be no restriction on products with LLRICE62 in Australia and New Zealand. Appropriate labelling would allow consumers wishing to avoid GM rice to choose products accordingly.

Industry: No restrictions on imports of rice products containing LLRICE62 as these foods would be compliant with the Code. Processors – broader availability of rice products. Manufacturers – broader market access and increased choice in raw materials for food manufacturing. Retailers – could offer broader range of rice products for sale. Exporters – possible costs if high-value export markets demanded certification on GM status of rice products, although this applies to both Options. Costs to food industry as some food ingredients derived from LLRICE62 would be required to be labelled as genetically modified.

Government: If LLRICE62 was detected in rice imports, approval would ensure compliance of those products with the Code which would reduce the potential for trade disruption on regulatory grounds. No potential conflict with WTO responsibilities. This option could impact on monitoring resources, as certain foods derived from LLRICE62 will be required to be labelled as genetically modified.

7.3 Comparison of Options

As food from glufosinate ammonium-tolerant LLRICE62 has been found to be as safe as food from conventional non-GM varieties of rice, option 1 is likely to be inconsistent with Australia's and New Zealand's WTO obligations.

Option 1 would also limit the range of imported rice products permitted in the Australian and New Zealand markets if GM rice is commercialised, as commingling of commercially harvested rice is common practice. The food industry and consumers could therefore experience some restrictions in the availability of imported rice products with Option 1.

Assuming that GM rice (LLRICE62) is eventually commercialised and enters international trade in rice products, industry costs associated with the requirement for quality assurance documentation would be independent of food approval in Australia and New Zealand. As well as the usual documentation on the source and nature of the rice, information on the GM status would also be required, whether or not LLRICE62 was approved in the Code.

LLRICE62 provides an agronomic benefit to primary producers that may result in lower production costs and higher yields, which could flow to other sectors downstream, including consumers in Australia and New Zealand, as lower food prices. This benefit would apply if LLRICE62 is commercialised and approved for growing by the relevant regulatory agencies. As the majority of foods derived from LLRICE62 would be required under the Standard to be labelled as genetically modified, Option 2 would accommodate consumers wishing to avoid GM rice as they would have adequate information on food labels to allow informed choice.

Given that the safety of LLRICE62 for human consumption has been adequately demonstrated, amending Standard 1.5.2 giving approval to food from glufosinate ammonium-tolerant rice LLRICE62 (Option 2) is the preferred option. With Option 2, the benefits (broader availability, consistency with WTO obligations, no trade disruption) outweigh the costs (possible restriction of choice for consumers wishing to avoid GM rice, labelling requirements for some rice products). Some potential costs apply to both options (certification of GM status, resource implications for government enforcement agencies).

Any sectors of the food industry or consumers wishing to avoid GM rice products will in general be able to identify products containing LLRICE62 due to mandatory labelling of foods where novel DNA and/or novel protein is present in the final food.

8. Limits on herbicide residues

Residues of any agricultural chemicals, for example herbicides, can only legally be present in food if the residues comply with Standard 1.4.2 – Maximum Residue Limits (Australia only). Standard 1.4.2 lists the maximum residue limits (MRLs) for agricultural and veterinary chemical residues present in food. According to the Standard: *If a maximum residue limit for an agricultural or veterinary chemical in a food is not listed in Schedule 1 there must be no detectable residues of that agricultural or veterinary chemical in that food. Also, if an agricultural or veterinary chemical is not listed in Schedule 1, there must be no detectable residue of that chemical and no detectable residue of any metabolites of that chemical in food.*

The MRL is the highest concentration of a chemical residue that is legally permitted or accepted in a food. The MRL does not indicate the amount of chemical that is always present in a treated food but it does indicate the highest residue that could possibly result from the registered conditions of use. The concentration is expressed in milligrams of the chemical per kilogram (mg/kg) of the food.

MRLs assist in indicating whether an agricultural or veterinary chemical product has been used according to its registered use and if the MRL is exceeded then this indicates a likely misuse of the chemical product. MRLs are also used as standards for international trade in food. In addition, MRLs, while not direct public health limits, act to protect public health and safety by minimising residues in food consistent with the effective control of pests and diseases.

Food products from conventional (non-GM) and GM crops alike must comply with Standard 1.4.2, including the MRLs in the Standard. Standard 1.4.2 includes MRLs for glufosinate ammonium residues in a number of agricultural products, including citrus fruits, berries, stone fruits, tomato, tree nuts and meat (mammalian). However, there is no MRL for glufosinate ammonium in rice products and therefore no detectable residues are permitted in rice or rice products, including imported rice products.

The *Agreement between the Government of Australia and the Government of New Zealand concerning a Joint Food Standards System* (the Treaty), excludes MRLs for agricultural and veterinary chemicals in food from the system setting joint food standards. Australia and New Zealand independently and separately develop MRLs for agricultural and veterinary chemicals in food. For New Zealand, maximum residue limits for agricultural compounds are included in the New Zealand (Maximum Residue Limits of Agricultural Compounds) Food Standards, 2007 (and subsequent amendments) issued under sections 11C and 11Z of the Food Act 1981.

The Trans Tasman Mutual Recognition Arrangement (TTMRA) between Australia and New Zealand commenced on 1 May 1998. The following provisions apply under the TTMRA.

- Food produced or imported into Australia that complies with Standard 1.4.2 can be legally sold in New Zealand.

- Food produced or imported into New Zealand that complies with the New Zealand (Maximum Residue Limits of Agricultural Compounds) Food Standard, 2007 can be legally sold in Australia.

9. Labelling of GM foods

Under Standard 1.5.2, all GM foods are required to be labelled if novel DNA and/or novel protein is present in the final food, or if the food has altered characteristics. Highly refined or processed foods that do not contain DNA or protein are exempt from labelling. This is because, unless there are compositional differences, these foods are indistinguishable from the conventional counterpart. In the case of foods derived from LLRICE62, a simple modification to the rice has not resulted in changes to composition and therefore labelling would be based only on the presence of novel DNA and/or protein, according to the Standard. These mandatory labelling requirements apply both to imported and domestically produced foods.

Novel protein (PAT) was detectable in the grain and therefore whole grain would be required to be labelled as genetically modified. In addition, it is anticipated that processing of rice into food fractions could result in the need to label as follows:

- rice flour – labelling required due to presence of novel DNA/protein;
- rice bran – labelling required due to presence of novel DNA/protein; and
- rice bran oil – labelling not required due to absence of detectable DNA or protein.

FSANZ has recently updated a User Guide (available on the FSANZ website) developed to assist industry with compliance with GM food labelling. It is the responsibility of the food business (manufacturer, supplier, importer, etc) applying the food label or selling the food, to meet the requirements of the Standard and ensure the accuracy of the label. The purpose of the Guide is to simplify the steps a food business should go through in order to interpret the Standard appropriately and apply the labelling requirements to their products.

Where a manufacturer chooses not to use ingredients from a GM source in a food, the Standard allows for not more than 10 g/kg (1%) per ingredient as unintended presence of a GM food. Verification of the non-GM source of the food or ingredient may include documents from identity preserved or other production systems that segregate or otherwise verify that the food is not of GM origin.

COMMUNICATION

10. Communication and Consultation Strategy

As normally applies to all GM food assessments, FSANZ has applied a communication strategy to this Application that involves advertising the availability of assessment reports for public comment in the national press and placing the reports on the FSANZ website for free public access. In addition, FSANZ issued media releases drawing journalists' attention to this Application. Two rounds of public comment have been conducted as part of the normal application process under the old procedures which were replaced by a new assessment process on 1 October 2007.

The Draft and Final Assessments are distributed directly to major stakeholders. The Applicant, and individuals and organisations that made submissions on this Application were notified at each stage of the Application. After the FSANZ Board has considered the Final Assessment Report, if the draft amendment to the Code is approved, the Board's decision will be notified to the Ministerial Council. If the approval of food derived from LLRICE62 is not subject to review, the Applicant and stakeholders, including the public, will be notified of the gazettal of changes to the Code in the national press and on the website. In addition, FSANZ provides an advisory service to the jurisdictions on changes to the Code.

11. Consultation

11.1 Public consultation

Two rounds of public consultation have been conducted on this Application during the statutory timeframe.

After Initial Assessment, public comment was sought between 13 December 2006 and 7 February 2007; eight submissions were received. Following release of the Draft Assessment, the Application was open for comment between 3 October 2007 and 14 November 2007; nineteen submissions were received. A summary of all submissions is included in **Attachment 3** to this Final Assessment Report.

The Australian rice industry is strongly opposed to the approval of LLRICE62 rice primarily on economic grounds. FSANZ has discussed trade and other market-related issues with other areas of government and with representatives of the rice industry in Australia as major stakeholders.

FSANZ has taken the submitters' comments into account in preparing the final assessment of this Application. Specific issues relating to food derived from LLRICE62 have been addressed in the report. The major issues raised in submissions are discussed in the following sections.

11.1.1 Potential impact on the Australian rice industry

The rice industry in Australia, including major stakeholders Ricegrowers Limited (trading as SunRice), Riviana Foods Pty Ltd, and an industry body, the Ricegrowers' Association of Australia, are strongly opposed to the Application. One basis for their opposition is the view that approval of GM rice could have a major adverse impact on their economic viability and international competitiveness by creating the need for additional compliance costs. These costs could be imposed on processors and exporters of rice processed in Australia, as well as manufacturers of rice products for the domestic market, and be passed on to ricegrowers.

It is claimed that a new compliance regime would be needed to satisfy certain export markets, particularly those that are intolerant of GM foods, that Australian rice products are non-GM. The regime could involve the need for expensive analytical testing of rice processed in Australia, and generating quality assurance documentation relating to GM status. These requirements would impose significant additional costs on the industry which is already operating under a weight of compliance testing for the absence of substances such as contaminants and agricultural chemicals in rice products exported from Australia.

In support of these claims, Ricegrowers Limited provided extensive information on the immediate trade effects resulting from the discovery in 2006 of the adventitious presence of an unapproved line of GM rice (LLrice601) in consignments of commercial long grain rice grown in the USA and destined for export markets in Japan and Europe.

Submissions from four Members of Parliament (Federal and State), the National Farmers' Federation (NFF) and the Australian Food and Grocery Council (AFGC) expressed support for those involved in the rice industry in Australia. They reiterated the claims and concerns of Ricegrowers Limited that approval of GM rice could pose a risk to the viability of their business through economic and trade impacts without providing any apparent benefit to Australian consumers. Rather than approve food derived from LLRICE62, the AFGC suggested that FSANZ consider approving only imported processed or manufactured food containing LLRICE62 or its derivatives, as an alternative regulatory measure. The NFF urged FSANZ to give greater consideration to the desirability of an efficient and internationally competitive food industry in Australia, while noting that it also supports the right of farmers to choose a method of production, including GM crops, best suited to their business needs.

11.1.1.1 Response

FSANZ acknowledges the predominantly economic concerns expressed by stakeholders involved in the rice industry in Australia. However, FSANZ does not agree with the industry's perceptions that approval of food derived from LLRICE62 in Australia and New Zealand, of itself, would be a major factor in any adverse effects on international trade or the domestic market in relation to rice products. Rather, FSANZ considers that approval of LLRICE62 is appropriate for the following reasons:

- commercialisation of GM rice anywhere in the world would be likely to result in the need for certification on the GM status of Australian rice products, either exported or sold on the domestic market, irrespective of whether GM rice is approved in Australia and New Zealand;

- the pre-market safety assessment conducted by FSANZ found no public health and safety concerns associated with food derived from LLRICE62;

- rejection of this Application without a supporting risk assessment could expose Australia and New Zealand to challenges in the WTO or potentially compromise other legitimate trade agreements;

- obtaining regulatory approval in a number of importing countries prior to commercialisation is appropriate and necessary to ensure no disruption to international trade;

- mandatory labelling for GM foods approved in the Code, means that the majority of rice products derived from LLRICE62 would be labelled as GM, allowing for consumer choice and for normal market forces to determine the extent of use of the products; and

- the majority of approved GM foods to date do not contain characteristics that provide direct benefits to consumers therefore LLRICE62 is not an exceptional case.

Further detailed consideration of these and other relevant issues is provided in the following sections.

Statutory obligations in assessing food derived from LLRICE62

FSANZ must consider the merits of this Application according to statutory obligations in our legislation. Section 18(1) of the FSANZ Act states that the objectives (in descending priority order) of the Authority in developing or reviewing food regulatory measures are:

(a) the protection of public health and safety; and
(b) the provision of adequate information relating to food to enable consumers to make informed choices; and
(c) the prevention of misleading or deceptive conduct.

In developing a food regulatory measure, FSANZ must also have regard to a number of other objectives listed in section 18(2) of the FSANZ Act. Several submitters urged FSANZ to consider one of these more fully, in particular, *the desirability of an efficient and internationally competitive food industry.*

In preparing this Final Assessment, FSANZ has considered the issues raised by major stakeholders in the Australian rice industry in light of our major objectives. Following an evaluation of a large amount of information supplied in support of their claims, FSANZ has concluded that the continued viability of the rice industry in Australia is not dependent on the single issue of an approval for LLRICE62 in the Code. FSANZ acknowledges that economic impacts could flow to the industry if and when GM rice is commercially produced, however this is likely to occur irrespective of any food approval in Australia and New Zealand. If commercialisation of LLRICE62 proceeds, the rice industry in Australia will need to adapt to changing market conditions in order to continue exports to other countries. Therefore FSANZ considers the decision to commercialise GM rice will be the overriding factor impacting on global trade in rice.

Overall, FSANZ considers that the benefits to international trade, the food industry, consumers and government from approving food derived from LLRICE62 clearly outweigh the costs to these sectors, particularly when approval is supported by the conclusions of the risk assessment.

Potential impact on the rice industry in Australia

FSANZ does not consider the adverse impacts on rice producers and exporters in the USA in 2006, following detection of unapproved GM rice in commercially produced non-GM rice, to be indicative of likely reactions in countries importing fully approved rice products.

The disruption to US trade occurred because of the unexpected presence of GM rice (predominantly LLrice601 but also LLrice06, LLRICE62, LLrice604 and others) in consignments of commercially grown long-grain rice destined for export. The detection of LLrice601 and other lines resulted in some trading countries requiring certification for the absence of GM rice in US rice exports. Importantly however, the necessity for testing of consignments was because, as GM products, the experimental varieties were subject to regulatory approval and did not have permission to enter the food supply.

According to US documentation, the company had obtained regulatory approval for LLRICE62 and a similar variety LLrice06 in 1999, however these varieties had never been sold to US rice growers. In contrast, LLrice601 and other similar GM varieties were not approved by the relevant US authorities, the Food and Drug Administration (USFDA) and the Department of Agriculture (USDA). Therefore any positive detection of these GM varieties in commercially grown non-GM rice was regarded as a contamination event. The company subsequently sought deregulation (approval) for LLrice601 towards the end of 2006, as a means of ensuring the safety of US long-grain rice for human consumption.

The USDA states that the US is one of the largest exporters of rice in the world, supplying approximately 13% of the world's rice trade; almost half of the US rice crop is exported annually. The marketing system used means that rice is harvested from thousands of farms throughout the US and commingled before being shipped through local, regional and terminal distribution centres. This system thus ensured that unapproved LLrice601 became widely distributed in long-grain rice consignments harvested over a certain period.

Immediately following the disclosure of this information, it is reported that Japan banned the import of long-grain rice from the US. In addition, the European Union demanded testing of shipments of long-grain rice from the US to certify that they were free of GM varieties.

It was also reported that Russia, South Korea, Mexico, Canada, the Philippines and Taiwan imposed testing requirements or halted the importation of US rice. There was therefore an immediate effect on trade and resulting legal implications for the developers. It is claimed that as a direct result of the adventitious presence of LLrice601, market perceptions and the value of US rice exports diminished.

The most significant factor in these market reactions is that LLrice601 was, at the time, not approved in the US, nor in any other trading country. FSANZ is aware that under EU food safety legislation, only GM organisms which have undergone a thorough scientific assessment and authorisation procedure may be put on the EU market. Countries such as Japan also have an established procedure for the pre-market evaluation of GM foods. Therefore, the market reactions in the EU and elsewhere would be expected based on the knowledge that any country would be likely to refuse imports that contravened their existing food regulations.

To avoid a similar experience in the future, the Applicant has therefore applied for pre-market approval of LLRICE62 in key export markets including in parts of Asia and the European Union, in addition to Australia and New Zealand. Approval as food has already been obtained in Argentina (2006), Canada (2006), Columbia (2007), Mexico (2007), the Russian Federation (2003) and the USA (2000). Only by seeking early regulatory approval in countries representing possible markets for US rice can the company ensure that the adverse trade impacts in 2006-2007 resulting from the presence of LLrice601 would not be repeated. Obtaining food approvals in a number of countries for LLRICE62 prior to commercialisation is therefore a necessary and appropriate course of action for the Applicant in order to build market and consumer confidence in the safety of the product.

In relation to possible reactions of certain markets in the Middle East, FSANZ is already aware of their cautious approach to GM foods. These views apply to all GM crops approved to date in Australia and New Zealand and in other parts of the world, and are therefore not unique to GM rice.

Overall, any impact on Australian businesses that trade in rice products may only be realised once commercial production of GM rice (LLRICE62) occurs in countries where it has been approved for growing. Following commercialisation, it is reasonable to assume that various markets would demand testing of rice products for compliance with their respective food regulations. Such additional compliance and certification costs could also apply to exports of Australian rice products regardless of a food approval for LLRICE62, even in the absence of an approval to grow the crop in Australia and New Zealand, because:

(i) approval for LLRICE62 in other countries and commingling practices mean that LLRICE62 it is more likely to be present in any imports of rice and processed rice foods; and
(ii) if unapproved in Australia but approved elsewhere, it is likely that testing and documentation would be required by export markets to ascertain the GM status of Australian rice products.

Decisions relating to the commercialisation of GM rice will be made by commercial seed companies involved in the rice industry who will decide on the market applicability of their products. Normal market forces will subsequently determine whether the product is commercially viable.

Approval of LLRICE62 however does not mean that all rice products would be derived from a GM source. Importers and manufacturers involved in the food industry in Australia and New Zealand would continue to be able to source non-GM whole rice or derivatives if preferred. Further, approval of LLRICE62 in Australia and New Zealand would not necessarily compromise trade in non-GM rice, particularly as there is no intention to cultivate GM rice in either Australia or New Zealand. In the event of international market penetration of GM rice, a price premium may apply to non-GM varieties.

Implications of commercialising GM rice

Assuming that the Applicant intends, at some point in the future, to commercialise LLRICE62, seeking food regulatory approval in Australia and New Zealand is prudent. Obtaining approval prior to growing GM rice on a commercial scale provides benefits for many sectors of the community. FSANZ has outlined these benefits in the Regulatory Impact Statement (RIS) in the Draft Assessment Report, however due to claims that these were insufficient to justify an approval, further analysis is provided here.

Approval of LLRICE62 in Australia and New Zealand would ensure that any rice products, imported from countries where LLRICE62 was approved and already in use in foods, would comply with the Code. Products with rice derivatives could involve a large number of processed foods including breakfast cereals, bakery products and confectionery. Approval for LLRICE62 would ensure continuing availability and trade in such products. In the absence of approval, certain products may not be permitted to enter the Australian and New Zealand markets. The lack of regulatory approval in our markets could therefore potentially adversely affect food importers, wholesalers and manufacturers who use imported raw ingredients, food retailers who stock imported products and domestic consumers.

Once LLRICE62 is commercialised, documentation relating to the GM status of rice products entering Australia and New Zealand as imported foods would be required. Rice imports would also be subject to random testing to ascertain their GM status and compliance with the Code. If LLRICE62 was not approved in the Code, Australia and New Zealand would be obliged to reject any rice products that did not comply with domestic food standards, even if LLRICE62 was produced lawfully in its country of origin. Disruption to trade in rice products could occur, as was the experience in 2006 in the European Union and Japan concerning the unapproved LLrice601 line.

The safety assessment found no public health and safety concerns in approving LLRICE62, therefore Option 1 (no approval for LLRICE62) is not supported by the conclusions of the risk assessment. Rejection of products from trading partners without a supporting risk assessment could expose Australia and New Zealand to challenges in the WTO, and could also potentially compromise other legitimate trade agreements. FSANZ therefore considers that Option 1 is associated with the potential to cause significant disruption to trade with concomitant adverse impacts on food producers, consumers and government, particularly enforcement agencies.

FSANZ acknowledges that Australian rice exports are currently not associated with GM products. However, this is primarily because GM rice to date has not been grown commercially anywhere in the world. Nevertheless, FSANZ monitors developments in plant biotechnology and is aware that advanced research in GM rice has been ongoing in a number of countries including China, India, the Philippines and the US for some time.

Documentation provided to FSANZ by Ricegrowers Limited confirms that the USA Rice Federation sought a commitment from biotechnology companies *to not commercialise GM rice until there is international regulatory acceptance and approval* from major importers of US rice. This position has been adopted specifically to avoid the detrimental effects on international trade in rice from the presence of unapproved lines of GM rice. While Australia and New Zealand are not major importers of US rice, seeking approval for LLRICE62 in Australia and New Zealand through this Application is compatible with a stepwise, systematic approach before commercialisation of the product, to ensure compliance with domestic food standards.

Finally, FSANZ does not support the AFGC suggestion for FSANZ to restrict a regulatory approval only to imported, processed foods containing LLRICE62. Such an approval would be inconsistent with the conclusions of the safety assessment and would be likely to lead to complexity in enforcement activities, particularly at the borders.

Consumer confidence in GM foods

Some submitters also claim that there is no consumer demand for LLRICE62 and that consumers are wary of GM foods in general. However, not approving LLRICE62 could be misconstrued by consumers as it would imply that the foods were not safe for human consumption. The scientific evidence supports the conclusion that food derived from LLRICE62 is as safe and wholesome as food derived from conventionally produced rice. Food regulatory agencies in Canada, the US and other countries have arrived at a similar conclusion based on an evaluation of the scientific evidence. In addition, the European Food Safety Authority (EFSA), responsible for food risk assessments for the European Union, has recently publicly released an evaluation of the safety of LLRICE62 and concluded that LLRICE62 is as safe as the non-GM comparator (EFSA, 2007[1]).

Mandatory labelling should provide consumers in Australia and New Zealand with sufficient information to enable informed choice at the point of sale. With the exception of rice bran oil, rice products derived from LLRICE62 would be required to be labelled due to the likelihood that the novel protein (PAT) would be present at detectable levels in the food. Moreover, as the majority of whole rice on the market in Australia is either domestically produced or sourced from Vietnam or Thailand, it is likely that most of these supplies would continue to be non-GM rice. As a result, if GM rice is approved, consumers will have information necessary for them to make an informed choice according to their preferences.

LLRICE62 contains one new gene that confers tolerance to glufosinate ammonium herbicides, an agronomic trait. As such, the planting of LLRICE62 may provide direct benefits to farmers in terms of improving weed management and reducing the use of other herbicides, thereby lowering overall production costs. While there are no direct nutritional benefits for consumers, lower production costs may be passed on to consumers indirectly through lower food prices. This applies to the majority of GM foods approved in the Code to date where an agronomic trait is present. Any direct benefits of the genetic modification flow primarily to growers rather than to consumers.

[1] 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., *The EFSA journal* (2007) 588, 1-25.

111.1.2 Enforcement costs

The NSW Food Authority reiterated comments relating to high enforcement costs for government in terms of testing of foods for compliance with Standard 1.5.2. The Authority advised that detection of GM organisms and their derivatives is more complex and expensive than other food analyses and the paper-trail is not reliable because it is not mandatory. Some jurisdictions have previously proposed that a national enforcement strategy for GM food approvals could be needed to address the rising costs associated with enforcement activities.

11.1.2.1 Response

The requirement for GM foods to be labelled as genetically modified if novel DNA and/or proteins are present in the final food was agreed to by the Ministerial Council on 24 November 2000 and came into effect on 7 December 2001. These labelling requirements were put in place at the request of the Ministerial Council, and following full consideration of an economic and financial assessment of the potential costs to both industry as well as governments.

A User Guide was subsequently developed by FSANZ, to assist industry to comply with the labelling requirements and to reduce the reliance on laboratory testing as the sole enforcement tool, as a means to reduce the overall cost burden on both industry and government. The User Guide outlines the type of documentation that would constitute compliance with the Standard, and notes that enforcement agencies can review these business documents as a first step in assessing compliance.

Costs to government enforcement agencies are likely to arise with the status quo if LLRICE62 is commercialised. In this situation, where LLRICE62 is commercially grown but no food approval is in place, compliance monitoring would be required to ensure rice products contain no GM components.

11.1.3 Assessment of novel protein

A submission from Greenpeace comments on a number of safety issues that are already addressed in the Safety Assessment (at Attachment 2) or elsewhere in this report. Greenpeace also contends that the studies on the novel protein, PAT, should have used the protein produced directly in the plant. They cite the study on the CSIRO GM peas conducted by the John Curtin School of Medical Research (JCSMR) as an example of this issue.

11.1.3.1 Response

Novel proteins are often expressed at low levels in transgenic plants. Expression levels of PAT in the food parts of the LLRICE62 plants, the grain in this case, represent approximately 0.02% of the crude plant protein. Due to the low level of expression in the plants, larger quantities of the PAT protein were produced in bacteria as reference material for subsequent use in toxicity and allergenicity studies. As these types of studies generally require larger amounts of protein, using bacterial expression systems is scientifically valid.

Demonstrating the equivalence of the novel protein produced in the plant and the reference material produced in the laboratory is a key component of the safety assessment.

Detailed analyses were conducted on the PAT protein produced in LLRICE62 and the microbially-produced PAT protein to demonstrate their structural, functional and biochemical equivalence. Details of the equivalence tests are reported in Section 4.3 of the safety assessment (Attachment 2).

As indicated in the Greenpeace submission, in 2005 the CSIRO discontinued a research project on GM peas following the publication of the results of an immunogenicity study in mice. Regulatory agencies such as FSANZ consider this action as evidence that subjecting potential GM products to comprehensive, pre-commercial testing is effective in identifying any unintended changes which may potentially have implications for the safety of the food. The peas were modified for protection against the pea weevil by introduction of an alpha amylase inhibitor gene (producing the α-AI protein) from green bean. The announcement by CSIRO was perceived by some groups to cast doubt on the rigour of the safety assessment used by FSANZ in the approval of GM foods. However, as the peas were still in the research phase, FSANZ had not yet had the opportunity to assess any of the safety data.

A number of studies were undertaken by CSIRO to characterize the α-AI protein expressed in the transgenic peas and compare it to the natively expressed α-AI protein in the common bean, including Western blot and MALDI-TOF mass spectrometry. Those studies indicated differences in glycosylation pattern between the two proteins. The demonstration of structural differences between the transgenic α-AI in pea and the form natively expressed in bean raised the concern that the modified form of α-AI expressed in peas may have enhanced immunoreactivity. This hypothesis therefore triggered a study which was undertaken at the JCSMR. The results indicated that the modified α-AI protein, and not the native form, predisposed mice to antigen-specific inflammation. The results of the study were published by CSIRO in the Journal of Agricultural and Food Chemistry on 16 November 2005.

The safety assessment protocol developed by FSANZ recognises the possibility of post-translational modification, and glycoprotein analysis is part of the battery of tests applied to the plant-produced protein. The safety of the PAT protein has been considered previously by FSANZ in assessing other glufosinate ammonium-tolerant crops and no post-translational modification of PAT has ever been detected. The results obtained in the characterisation of the PAT protein produced in LLRICE62 show that it is equivalent to the microbially-produced protein based on comparable electrophoretic mobility, enzyme activity, immunoreactivity and absence of detectable glycosylation.

11.1.4 Potential allergenicity of GM foods

The submission from the Physicians and Scientists for Responsible Genetics (PSRG, New Zealand) expresses the view that all GM foods are inherently unsafe and considers that the potential to generate a new food allergen has not been adequately tested in humans. The PSRG claim that reported increases in food allergies to soy foods occurred just after GM soy was introduced, and this could be due to the novel protein being an allergen.

11.1.4.1 Response

The safety assessment considers the potential for the novel protein to be toxic or allergenic in humans by evaluating a raft of physical, biochemical, bioinformatic and animal toxicity studies conducted specifically on the novel protein.

The assessment also considers the history of use of the host plant and any known allergenicity associated with the source of the novel gene.

Concerning this Application, the PAT protein has been assessed for potential toxicity and allergenicity on multiple occasions by FSANZ. It is used in a number of approved GM crops with tolerance to glufosinate ammonium herbicides and has therefore been in the food supply now for 5-10 years. FSANZ is confident that the PAT protein is not associated with any adverse impact on human health. The scientific evidence shows that PAT would be digested in humans like the majority of all other dietary protein from plant and animal sources.

In addition, dietary exposure to PAT through consumption of foods derived from LLRICE62 would be expected to be very low, despite rice being a staple food for sectors of the population. In some processed rice fractions, plant proteins including PAT would be removed entirely.

FSANZ does not consider the potential allergenicity of soy products to be relevant to the assessment of food derived from LLRICE62. Soybean is one of a group of eight commonly consumed foods responsible for over 90% of all true food allergies (WHO[2]); other allergenic foods in the group include peanuts, milk, cereals (wheat), fish, shellfish, eggs and tree nuts. There are reports of increasing levels of food allergies in humans however it is not clear whether this may be due to increased exposure to allergenic foods, increased clinical reporting, or a combination of causes. While some groups, opposed to the use of biotechnology in food, speculate that the increase may be associated with the introduction of GM foods, after 10-15 years of widespread use, there is no substantiated, documented evidence of approved GM products being associated with food allergies.

11.2 World Trade Organization (WTO)

As members of the World Trade Organization (WTO), Australia and New Zealand are obliged to notify WTO member nations where proposed mandatory regulatory measures are inconsistent with any existing or imminent international standards and the proposed measure may have a significant effect on trade.

Guidelines for assessing the safety of GM foods have been developed by the Codex Alimentarius Commission and have the status of standards for WTO purposes. An amendment to the Code to allow food derived from LLRICE62 may be of interest to other WTO member nations because it pertains to the safety of GM food and is likely to have a liberalising effect on international trade. For these reasons, notification was recommended to the agencies responsible in accordance with Australia's and New Zealand's obligations under the WTO Sanitary and Phytosanitary Measure (SPS) Agreements. This enabled other WTO member countries to comment on proposed changes to standards where they may have a significant impact on them. No submissions were received under this notification.

[2] World Health Organisation INFOSAN Information Note No. 3/2006 – Food Allergies, 9 June 2006.

CONCLUSION

12. Conclusion and Decision

Decision

Amend Standard 1.5.2 – Food produced using Gene Technology, to include food derived from glufosinate ammonium-tolerant rice LLRICE62 in the Table to clause 2.

Reasons for Decision

An amendment to the Code approving food derived from glufosinate ammonium-tolerant rice LLRICE62 in Australia and New Zealand is approved on the basis of the available scientific evidence, for the following reasons:

- the safety assessment did not identify any public health and safety concerns associated with the genetic modification used to produce glufosinate ammonium-tolerant rice LLRICE62;

- food derived from glufosinate ammonium-tolerant rice LLRICE62 is equivalent to food from the conventional counterpart and other commercially available rice varieties in terms of its safety for human consumption and nutritional adequacy;

- labelling of certain food commodities derived from glufosinate ammonium-tolerant rice LLRICE62 will be required if novel DNA and/or protein is present in the final food; and

- a regulation impact assessment process has been undertaken that also fulfils the requirement in New Zealand for an assessment of compliance costs. The assessment concluded that the preferred option is option 2, an amendment to the Code.

13. Implementation

It is proposed that the draft variation come into effect on the date of gazettal.

ATTACHMENTS

1. Draft variation to the *Australia New Zealand Food Standards Code*
2. Safety Assessment Report for glufosinate ammonium-tolerant rice LLRICE62
3. Summary of public submissions

ATTACHMENT 1

Draft variation to the *Australia New Zealand Food Standards Code*

Standards or variations to standards are considered to be legislative instruments for the purposes of the Legislative Instruments Act (2003) and are not subject to disallowance or sunsetting.

To commence: on gazettal

[1] ***Standard 1.5.2*** *of the Australia New Zealand Food Standards Code is varied by inserting in the* Table to clause 2 –

Food derived from glufosinate ammonium-tolerant rice line LLRICE62	

Attachment 2

SAFETY ASSESSMENT

SUMMARY AND CONCLUSIONS

Background

A new trait has been introduced into medium-grain rice (*Oryza sativa*) used for production of a wide range of food products. Known as LLRICE62 (LibertyLink® rice), this line has been genetically modified (GM) for tolerance to broad-spectrum herbicides containing glufosinate ammonium as the active ingredient. LLRICE62 has been developed for commercial cultivation in rice-growing regions of the United States, and could enter the Australian and New Zealand food supply through imports of rice products.

Glufosinate ammonium (or phosphinothricin, L-PPT) is a non-selective, contact herbicide that provides post-emergence control of many broadleaf and grassy weeds. The mode of action of the herbicide is to inhibit the activity of glutamine synthetase, an essential enzyme involved with nitrogen metabolism in plants. The inhibition of glutamine synthetase results in an over accumulation of ammonia in cells, which typically leads to plant death. In LLRICE62, the glufosinate ammonium-tolerant trait is achieved by insertion of the *bar* gene from *Streptomyces hygroscopicus* into the rice genome. This gene expresses the enzyme phosphinothricin acetyltransferase (PAT) which chemically inactivates the herbicide. The production of PAT by LLRICE62 enables the post-emergence use of glufosinate ammonium herbicides without adverse effects to the crop.

In conducting a safety assessment of food derived from LLRICE62, a number of criteria have been addressed including: a characterisation of the transferred gene, its function and stability in the rice genome; the changes to the rice at the level of the DNA and protein particularly in the edible portions of the plant; detailed compositional analyses; and the potential for the newly introduced protein to be either allergenic or toxic in humans.

This safety assessment report addresses the safety and nutritional impact of LLRICE62 when consumed as food. It does not address: potential environmental risks related to the environmental release of GM plants used in food production; the safety of animal feed or animals fed with products derived from GM plants; the safety of GM plants used in herbal supplements; or the safety of food derived from the non-GM (conventional) plant.

History of Use

Rice is a staple food for about half of the world's population. The predominant species *Oryza sativa* is grown worldwide; many different cultivars have been developed for diverse agricultural conditions. The morphology, physiology, agronomy, genetics and biochemistry of this species have been intensively studied over a long period.

The *bar* gene from *S. hygroscopicus*, a soil bacterium, confers tolerance to glufosinate ammonium when expressed in plants. The safety of GM crops containing the *bar* gene has been assessed previously by FSANZ. Numerous glufosinate ammonium-tolerant lines of canola, cotton and soybean expressing this bacterial gene are approved in the Code.

Description of the Genetic Modification

The combined results from the molecular characterisation of LLRICE62 confirm the presence of one functional intact copy of the *bar* gene inserted at a single locus in the rice genome. LLRICE62 does not contain any additional DNA elements other than those expected from the insertion of the transferred DNA. Fragments corresponding to partial genes, regulatory elements or additional vector backbone sequences were not detected. No marker genes encoding antibiotic resistance are present in LLRICE62. DNA sequencing has confirmed that no changes to the inserted DNA were introduced during the transformation process.

As a complete sequence of the *Oryza sativa* genome has been published, detailed bioinformatics studies of the region surrounding the inserted DNA were possible. The site of integration of novel DNA in LLRICE62 was found to be located on chromosome 6 in a region of repeat elements which make up more than 35% of the rice genome.

Characterisation of Novel Protein

LLRICE62 is tolerant to glufosinate-ammonium through the expression in the plant of the bacterial enzyme PAT. This enzyme chemically inactivates the herbicide by acetylation of the free amino group to generate the metabolite N-acetyl-L-PPT, which is unable to bind to the plant glutamine synthetase.

The PAT protein is expressed in LLRICE62 at very low levels in the unprocessed grain. When grown under normal field conditions including treatment with glufosinate ammonium, PAT constitutes 12.1 µg/g fresh weight in grain which corresponds to about 0.02% of the crude protein. PAT was detected at low levels in all processed commodity fractions derived from the grain, with the exception of rice bran oil which contains no plant proteins.

Assessment of potential toxicity and allergenicity

The potential toxicity and allergenicity of the PAT protein has been assessed previously by FSANZ and no safety concerns have been identified. No adverse effects were identified in acute toxicity studies in mice using purified PAT protein. The PAT protein does not exhibit sequence similarity with known protein toxins or allergens, and is degraded in conditions that mimic human digestion. Based on bioinformatic, biochemical and acute toxicity studies, PAT is considered non-toxic to humans and is unlikely to be allergenic. Similarly, reviews of the safety of the metabolites resulting from the inactivation of glufosinate-ammonium by PAT have concluded that the metabolites are less toxic or equivalent in toxicity to the parent compound in humans.

Compositional Analyses

Compositional studies were conducted over different seasons and environments to establish the nutritional adequacy of LLRICE62 and compare it with the conventional parental line and other commercial rice varieties under typical cultivation conditions.

The constituents measured were proximates (crude protein, fat, fibre, ash and moisture), amino acids, fatty acids, vitamins, minerals, and a small number of anti-nutrients relevant to rice grain.

No differences of biological significance were found between LLRICE62 and the conventional counterpart variety. Small differences in some nutrients were noted however the changes were not consistent across trial sites and do not indicate an overall pattern of change that could be attributed to the genetic modification. Based on the high degree of similarity in composition between LLRICE62 and conventionally produced rice varieties, no food safety issues were identified.

Nutritional Impact

The detailed compositional studies are considered adequate to establish the nutritional adequacy of the food and indicate that LLRICE62 rice is equivalent in composition to conventional rice varieties. Results from two feeding studies, one in growing-finishing swine and the other in broiler chickens, further support the data demonstrating that LLRICE62 is nutritionally equivalent to its conventional counterpart. Animals fed diets containing LLRICE62 were able to grow normally and produce food products with qualities and characteristics typical of animals fed on conventional diets. The introduction of products derived from LLRICE62 into the food supply is therefore expected to have minimal nutritional impact.

Conclusion

No potential public health and safety concerns have been identified in the comprehensive assessment of glufosinate ammonium-tolerant rice LLRICE62. On the basis of the data provided in the Application, and other available information, food derived from LLRICE62 is considered as safe and wholesome as food derived from the conventional parental line and other commercial varieties of rice.

1. INTRODUCTION

Rice has been genetically modified (GM) for tolerance to the broad spectrum herbicide glufosinate ammonium. The variety is known as LibertyLink® rice event 62 or LLRICE62, produced by Bayer CropScience Pty Ltd. The Applicant is seeking approval for this line of rice in the major rice producing countries around the world. Once appropriate regulatory approval has been obtained and the line is grown commercially, LLRICE62 could enter the Australian and New Zealand food supply through imported rice based foods and possibly as various forms of grain including milled and broken rice. Processed rice fractions include rice starch, flour, bran and bran oil.

Glufosinate ammonium (also referred to as phosphinothricin, L-PPT) is a non-selective, contact herbicide that provides post-emergence control of many broadleaf and grassy weeds. LLRICE62 is tolerant to glufosinate-ammonium through the expression in the plant of the bacterial enzyme phosphinothricin acetyl transferase (PAT) encoded by the *bar* gene from the soil bacterium *Streptomyces hygroscopicus.* The PAT enzyme chemically inactivates the herbicide. Expression of this enzyme in LLRICE62 therefore enables the use of glufosinate ammonium herbicides on post-emergence weeds, without adverse effects to the crop.

Glufosinate-ammonium is currently registered in Australia under the commercial name of Basta® for non-selective uses, or Finale® for turf and home garden uses, and as Buster® in New Zealand.

2. HISTORY OF USE

2.1 Donor Organisms

Streptomyces hygroscopicus

The source of the *bar* gene is the bacterial species *Streptomyces hygroscopicus*, strain ATCC21705 (Murakami *et al*., 1986). The *Streptomycetae* bacteria were first described in the early 1900's. These organisms are generally soil-borne, although they may also be isolated from water. They are not typically pathogenic to animals or humans, and few species have been shown to be phytopathogenic (Bradbury, 1986; Kutzner, 1981). Although these organisms are not used in the food industry, the *bar* gene from *S. hygroscopicus*, has been used to confer glufosinate ammonium tolerance in food producing crops including GM cotton (derived from strain ATCC21705) and GM hybrid canola, which are approved in Australia and New Zealand.

Cauliflower mosaic virus

The expression of the *bar* gene in LLRICE62 is controlled by the *35S* promoter and *35S* terminator derived from the cauliflower mosaic virus (CaMV). CaMV is a double stranded DNA caulimovirus with a host range restricted primarily to cruciferous plants.

The *35S* promoter and terminator elements from CaMV are used extensively to express introduced genes in plants and are well described in the literature. Only a defined, single DNA fragment of the CaMV genome corresponding to either the promoter or terminator has been used to construct the gene cassette inserted into the rice.
CaMV is not used in the food industry, however certain vegetables, notably the *Brassica* species, can be infected with this plant virus and may be consumed.

2.2 Host organism

Rice is the common name for the plant *Oryza sativa* L. which has a long history of use as food dating back at least 4000 years. Rice is used in various forms including whole and milled grain, flour and bran. The husks may be used for fertilisers and animal feed as well as for fibre production. Numerous varieties of rice have been developed from subspecies *indica*, *japonica* and *javanica.*

Rice is a staple food for half of the world's population with annual harvests of around 530 million tons. Over 90% of this production is from Asia, with around 5% from the Americas, 3% from Africa and another 1% from Europe and Oceania. The crop is well adapted to diverse growing conditions from cool climates to deserts (with irrigation) and is able to perform well in areas with saline, alkaline or acid-sulphate soils.

Rice is commonly consumed in Australia and New Zealand. It is typically cooked prior to consumption as parboiled rice, a milled grain or as a processed fraction.

3. DESCRIPTION OF THE GENETIC MODIFICATION

3.1 Transformation Method

The parental rice cultivar used for the transformation was Bengal, a medium grain rice variety adapted for the Southern United States (Linscombe *et al*. 1993. LSU Ag Center, Publication B-837).

The method used to transform the parental rice was the particle bombardment method, which involved direct transfer of a purified DNA fragment corresponding to the *bar* gene cassette (1502 base pairs, bp) which had been constructed in plasmid vector pB5/35Sbar (4161 bp). Cells that received and incorporated the introduced DNA and expressed the *bar* gene were selected on tissue culture media containing phosphinothricin (5 mg/L). These cells were allowed to develop into transgenic callus, which was transferred to regeneration medium where shoot and root development was induced. Seedlings were subsequently transferred to soil, placed in the greenhouse, and allowed to flower and set seed. Seed families were evaluated and, on the basis of the research results, transformation event LLRICE62 was selected for further development. The transformation was confirmed phenotypically by glufosinate ammonium application to leaves, and analytically by phosphinothricin acetyl transferase activity assay, and by PCR and Southern blot analyses (see Section 3.4).

3.2 Genetic elements in vector

Plasmid vector pB5/35Sbar was developed in a series of laboratory manipulations using *Escherichia coli* as the production organism. The vector is a derivative of pUC19 in which the β-lactamase gene was replaced with the *npt*III gene from vector pBIN19. To obtain the transforming DNA, the plasmid was digested with appropriate restriction enzymes, and the resulting restriction fragments were separated by gel electrophoresis.

A 1502 bp fragment containing the bar gene cassette P35S-*bar*-T35S was purified from the gel (refer to Table 1). The *npt*III gene was not included in the transforming DNA fragment.

Table 1: Genetic elements in plasmid pB5/35Sbar; size and function of elements in transforming DNA

Position	Size (bp)	Genetic Element and Function
0001 - 1025		Sequence from pBIN19 (Bevan, 1984) containing *npt*III gene (coding sequence is from 172-966).
1026 - 2195	*	Sequence derived from pUC19 (Yanisch-Perron *et al*., 1985).
2196 - 2204	8	Synthetic polylinker sequence
2205 - 2398	193	Complement of 35S terminator (**T35S**) from CaMV (Franck *et al*., 1980; Pietrzak *et al*., 1986), which terminates transcription and directs polyadenylation of the mRNA.
2399 - 2417	18	Synthetic polylinker sequence
2418 - 2969	551	Complement of ***bar*** gene from *Streptomyces hygroscopicus*, strain HP632 (Thompson *et al*., 1987), which encodes the PAT enzyme.
2970 - 2985	15	Synthetic polylinker sequence

Position	Size (bp)	Genetic Element and Function
2986 - 3517	531	Complement of the 35S promoter (**P35S**) from CaMV (Franck *et al*., 1980; Pietrzak *et al*., 1986), which directs high level constitutive expression in plants.
3518 - 3730	*	Sequence derived from pUC19
3731 - 3791		Synthetic right border fragment (RB) of the *Agrobacterium tumefaciens* octopine plasmid (Gielen *et al*., 1984).
3792 - 4161		Sequence derived from pUC19

* The transforming DNA is defined by a specific restriction enzyme site within this segment.

3.3 Function and regulation of novel genes

The only novel gene introduced into LLRICE62 is *bar*. This gene encodes the bacterial enzyme PAT, which confers resistance in the rice plant to the normally phytotoxic activity of glufosinate ammonium, the active ingredient in commercial herbicide preparations with the commercial names Basta® or Finale® in Australia, or Buster® in New Zealand. The promoter used to drive expression of PAT is derived from the cauliflower mosaic virus (CaMV), a common plant virus used widely for high-level constitutive expression of novel genes in plants.

3.4 Characterisation of the genes in the plant

Studies submitted:

1. Scott, A.. Molecular Characterisation of Glufosinate-tolerant Rice Transformation Event LLRICE62. Sponsor: Bayer CropScience Regulatory Affairs and Biotechnology, USA, Report No. OS 24 v2, completed August 2006.
2. De Beuckeleer, M. and Van der Klis, R.J.. Summary document molecular characterisation of glufosinate-tolerant rice transformation even LLRICE62. Report No. LLRICE62 SUM01, completed November 2004.
3. Van Herck, H., Habex, V. and De Beuckeleer, M.. Molecular characterisation of *Oryza sativa* transformation event LLRICE62. Report No. LLRICE62 MA-02, completed November 2004.

Integrity of the introduced gene cassette

Analysis of the DNA introduced into LLRICE62 was undertaken using a range of established molecular techniques. Southern hybridisation blots were performed on genomic DNA extracted from leaf tissue from LLRICE62 and non-transformed control rice plants to assess the following:

(i) number of insertions of the integrated expression cassette;
(ii) number of copies of the integrated expression cassette;
(iii) integrity of gene expression cassette;
(iv) absence of plasmid vector backbone; and
(v) stability of the inserted DNA with conventional breeding over several generations.

Total genomic DNA from LLRICE62 and control plants (var. Bengal) was extracted from the leaves of plants grown at the same time in the greenhouse. The presence of the introduced trait in LLRICE62 plants was confirmed by a standard glufosinate-ammonium dot identification assay.

The DNA samples were digested with a number of restriction endonucleases for use in the Southern blots. DNA from the pB5/35Sbar vector, containing the *bar* coding sequence, was used as reference material. For a positive control, digested genomic DNA prepared from the non-transgenic parental line was supplemented with approximately one copy of digested plasmid. This control was used to demonstrate that the experimental conditions allowed hybridisation of the probe with target sequences. The probe corresponded to the full-length inserted DNA segment (1502bp). The resulting pattern and molecular size of bands were analysed against the known number of specific restriction enzyme sites within the *bar* gene cassette. The number and pattern of bands obtained was consistent with the presence in LLRICE62 of one copy of the gene cassette used in the transformation. The results indicate also that the arrangement of genetic elements in the plant correlates exactly with those present in the transforming DNA segment.

Southern blot hybridisation of genomic DNA from LLRICE62 and the vector DNA was also performed in order to demonstrate the absence in the plant of any unintended sequences derived from the plasmid pB5/35Sbar. The blot was probed with a 2665 bp fragment corresponding to the remaining vector sequences outside of the gene cassette used in the transformation. Wildtype Bengal DNA samples were used as negative controls and wildtype plus one copy of pB5/35Sbar used as a positive control. Using the same conditions as in the previous experiments, additional vector sequences were not detected in either the transformed rice or the non-transformed negative control (as expected). The expected size fragments were detected in positive control samples. These results indicate that neither the *npt*III gene nor the bacterial origin of replication is present in LLRICE62.

Polymerase chain reaction (PCR) was used to further characterise the introduced DNA. The amplification strategy was to generate two overlapping fragments corresponding to the complete insert of event LLRICE62 using two sets of oligonucleotide primer pairs. One primer in each pair annealed to plant genomic DNA either upstream or downstream of the introduced DNA, and was paired with an insert-specific primer. The PCR amplifications generated DNA products of the expected sizes. The results of DNA sequencing of these products in both directions show that the insert in LLRICE62 is identical to the corresponding sequence in the transforming DNA segment.

The DNA sequence at the junction regions with flanking plant genomic DNA was determined to further analyse the insertion locus and also to investigate the possibility of expression of open reading frames (ORFs) created by the insertion of the 35S-*bar*-35T cassette. The ORF analysis provides information on whether any chimeric proteins arising from the insertion would be likely to be expressed. The 3' flanking sequence spanned 149 bp of rice genomic DNA, while the 5' flanking sequence consisted of 669 bp of genomic DNA. Visual examination of the sequences revealed short oligonucleotide repeats which were G-rich at the 3' end and somewhat T-rich at the 5' end, suggesting a region of low complexity (non-coding region). Further bioinformatics analysis using information on rice genomic sequences in various databases indicate that the insertion site is not a functioning gene.

Approximately 20 kb of sequence centred on the transgene insertion site was analysed for the presence of genes by the de novo gene prediction programme FGENESH. This software allows multiple gene finding on both strands. It predicts genes by predicting statistical differences between intron and exon sequence, the presence of consensus splice sites and transcription-related signals such as the presence of a transcriptional start signal and a polyadenylation site.

These bioinformatics analyses together with the Northern blot analyses do not indicate expression of any chimeric proteins arising from the insertion of the transgene in LLRICE62.

Location of the inserted DNA segment

Current molecular and bioinformatic techniques were used to characterise the chromosomal location of the insert DNA in LLRICE62 as far as possible. Two flanking sequences were analysed as if joined, to provide information about the (presumed) pre-insertion locus with a view to identifying any endogenous genes adjacent to the inserted DNA. The query sequence was subjected to a sequence similarity search using the BLAST algorithm (version 2, National Centre for Biotechnology Information, NCBI).

The complete sequence and assembly of the rice genome has been published, and this information was used to assist with the analysis. Alignments were examined against the presumed pre-insertion sequence and the PAC clone AP003539 (173301 bp) was identified. Apart from a deletion of 18 bp precisely at the 5' and 3' insertion boundaries, the PAC clone was an identical match with the flanking sequence identified in LLRICE62. It was therefore concluded that the insertion site of the 35S-*bar*-35T cassette in LLRICE62 is on chromosome 6. A number of other less perfect matches were found on the same chromosome as well as other rice chromosomes, suggesting repetitive sequences in the non-coding part of the genome.

From analysis of the rice genome already completed, it is known that repetitive sequences make up more than 35% of genomic DNA. Repeat elements may be simple, short repeats or longer, complex repeats and may be present in up to thousands of copies in the plant genome. The identity of the repeat element was verified using RepeatMasker2. This algorithm is an advanced programme used to detect and mask out repeated regions of genomic DNA for example before BLAST analysis. Using RepeatMasker2, it was found that the insertion site in LLRICE62 is not a functioning gene, but rather a repetitive element. RepeatMasker2 recognises a number of species-specific classes of repeated sequences and can be used to localise and identify repeats in any DNA sequence. Analysis of the 5' and 3' flanking sequences in LLRICE62 showed the presence of a MERMITE-18 repeat element, a short DNA transposon-like element present in thousands of copies in the rice genome, including copies in expressed genes.

Analysis of genomic region surrounding the transgene

The production of unexpected chimeric proteins as a result of transgene insertion is of particular relevance to food safety. In cases where there is 100% molecular identity between the transforming DNA and inserted DNA in the plant, and all regulatory elements including termination and polyadenylation signals are intact, there is little likelihood of forming unintended gene fragments that are transcriptionally active, and even less likelihood that a chimeric protein would be produced. In the case of glufosinate ammonium-tolerant LLRICE62, the transformation event has not resulted in any additions, deletions, rearrangements or partial insertions of the gene of interest, or its regulatory elements, as determined by the Southern blot, PCR analyses and direct DNA sequencing of the entire insert region. The Applicant nevertheless provided a bioinformatic evaluation of DNA sequences flanking the junctions of the inserted DNA in LLRICE62.

A gene prediction programme known as FGENESH was used for gene structure prediction (Softberry Inc.). It allows multiple gene finding on both strands of the DNA. FGENESH predicts genes by predicting statistical differences between intron and exon sequence, the presence of consensus splice sites and transcription-related signals such as the presence of a transcriptional start signal and a polyadenylation site.

Approximately 20 kb of sequence centred on the transgene insertion site was analysed for the presence of endogenous genes. Using the de novo gene prediction software, two flanking genes were predicted to lie on the opposite strand of the chromosome to the transgene cassette. These genes correspond to known, fully sequenced ESTs, AKD65054 and AK107459. The exact positions and orientations of the exons of the ESTs with respect to the originally sequenced transgene flanking regions were determined. The results indicate that the transcriptional regulatory sequences of these two genes are sufficiently distant from the insertion site to be unaffected by the insertion of the novel gene cassette 35S-*bar*-35T. In addition, the presence of these two native genes within this region of the chromosome makes it statistically unlikely that another endogenous gene is present in the region surrounding the transgene insertion.

Northern blot analysis

Northern blot analysis was performed on different plant tissues to (i) determine levels of expression in different parts of the plant, and (ii) detect any cryptic transcription arising from the insertion of the novel gene cassette and its junction with flanking plant DNA. Cryptic expression analysis is done to address the potential for unintended effects as a result of the gene insertion. For example, Northern analyses can be used to detect any expression of transgene and flanking sequences as open reading frame (ORF) fusions to investigate the possibility for generating novel hybrid proteins.

Expression of the transgene in various plant tissues was detected using a probe corresponding to the antisense bar gene sequence. The analysis demonstrated that the bar gene sequence present in LLRICE62 is expressed in leaf, stem, root and seeds of the plant, with seed showing the lowest levels of expression (about 10 fold lower than the other tissues). Additional Northern blot results, using RNA probes of flanking sequences, did not show any cryptic expression of the transgene sequence.

3.5 Stability of the genetic changes

Southern blot analysis was used to investigate the stability of the genetic modification in LLRICE62 over different generations. T2 and T3 seed from plants grown in the greenhouse was tested by a glufosinate dot identification assay to confirm the presence of the PAT protein. Genomic DNA was prepared from the T2 and T3 generations, and analysed under similar conditions used previously to characterise the transformation event. The conventional Bengal variety was used as a wildtype control. The results show that the number and size of fragments detected was as expected from the original Southern blot data, indicating that the event is stable at the genomic level over several generations.

In addition, the same type of analysis was performed on plants from three generations grown at different field locations in the USA, under different environmental conditions. The tested generations were grown in Puerto Rico (T3 plants), Louisiana (T5 plants) and Texas (T6 plants), and the same experimental conditions were applied.

Using the 1502 bp gene cassette as a probe, the pattern of fragments detected by Southern blots of these plants was the same as previously detected. The fragments correspond to the junctions between the inserted DNA and the flanking plant DNA on both sides, and therefore demonstrate the stability of the inserted gene cassette over multiple generations and in different field locations.

In addition, the expression of the PAT protein in grain from LLRICE62 was evaluated in two successive years (1998 and 1999) across multiple locations using a quantitative enzyme linked immunosorbent assay (ELISA). These results showed that PAT constituted 0.017% and 0.014% of the crude protein in successive years, indicating that the genetic modification in LLRICE62 is stable at the phenotypic level over time.

Stability of the inserted DNA in different genetic backgrounds

Transformation procedures lead to integration of DNA segments with unique flanking sequences that will not be altered by conventional crossing. To test the stability of the insertion event in LLRICE62 (Bengal variety), plants from this event were backcrossed using conventional breeding to several individual plants representing four rice varieties with distinctive genetic backgrounds: Bengal (medium-grain tropical Japonica), Cocodrie (long-grain tropical Japonica), Koshihikari (short-grain, temperate Japonica), and Teqing (short/medium-grain Indica background). The genomic DNA from the progeny of these crosses was analysed by Southern blot hybridisation in the same manner as before. The results obtained from this experiment showed that the number and size of fragments detected in all progeny was the same as in previous experiments. The insertion event in LLRICE62 appears to be stable at the genomic level when crossed into rice varieties with different genetic backgrounds.

3.6 Antibiotic resistance genes

The molecular characterisation shows that only the purified DNA fragment comprising the *bar* gene cassette was integrated into the rice genome during transformation. The bacterial selectable marker gene, *npt*III (which confers resistance to the antibiotics Kanamycin, Neomycin and GentamycinB) located on the plasmid backbone was not transferred to the plants. The absence of the bacterial marker gene in LLRICE62 was confirmed by Southern hybridisation analysis using a probe for the *npt*III gene.

3.7 Breeding history

Using the gene cassette described, a number of independent transformation events in rice were generated in 1997. Selection of the event designated as LLRICE62 was accomplished from assessment of field tolerance to glufosinate ammonium and agronomic performance across several generations. T_1 generation seed, harvested from self-pollinated T_0 plants surviving a herbicide tolerance screen in the greenhouse, were field planted in December 1997 (Puerto Rico winter nursery). Surviving T_1 plants were selected following glufosinate ammonium herbicide application. Panicles were harvested from individual plants and T_2 panicle rows were planted in May 1998 in Louisiana. Each row was planted with the seed from a single panicle.

Spraying with glufosinate ammonium herbicide was used to score the rows for segregation analysis of the phenotype. Rows containing no herbicide-sensitive plants were considered to be homozygous for the *bar* gene. Rows showing only partial resistance were considered to be segregating for the herbicide tolerance trait and containing homozygous and hemizygous surviving plants. In this situation, Mendelian inheritance for a single gene locus would predict one fully resistant row for every two partially resistant rows. The results of the analysis of four populations of T_2 panicle rows showed the expected ratio 1:2 was found with a high degree of certainty (Chi square test).

The fully resistant rows were harvested as independent populations for advanced variety evaluation. Selected T_3 generation panicles of the fully resistant rows were taken to the winter nursery in Puerto Rico in 1998 for seed increase to supply T_4 generation seed for multi-state evaluations (subsequently conducted in 2000). Each panicle-row was increased as an independent line and best performing lines were selected for further evaluation. These lines were used in breeding programs to produce new rice varieties by conventional crossing and selection.

4. CHARACTERISATION OF NOVEL PROTEINS

4.1 Function and phenotypic effects

Expression of the PAT protein in LLRICE62 plants confers tolerance to the herbicide glufosinate ammonium. The field performance criteria for glufosinate ammonium-tolerant rice varieties requires plants to be tolerant to the herbicide in the vegetative stages of rice plant development, spanning the rice plant growth stages of first leaf to panicle initiation. Herbicide applications are recommended for the rice plant growth stages of 2-4 leaf and first tiller. The leaves (blade and sheath) of the rice plant are the principle plant parts exposed to herbicide applications and therefore commercial-level herbicide tolerance depends on the function of the PAT enzyme in the leaves. No other novel proteins have been introduced into LLRICE62.

The mode of action of the herbicide

Glufosinate-ammonium (or phosphinothricin, L-PPT) is a potent inhibitor of the enzyme glutamine synthetase (GS) in both bacteria and plants. GS is an essential enzyme in nitrogen metabolism and amino acid biosynthesis in plants; it catalyses the conversion of glutamate and ammonia into glutamine, an essential amino acid used in many anabolic processes. The herbicide binds competitively to the enzyme by displacing L-glutamate from the active site (Thompson *et al.*, 1987). This binding blocks GS activity which results in the over-accumulation of ammonium ions and a decrease in glutamine. Inorganic ammonia, although a plant nutrient and metabolite, is toxic in excess and causes the inhibition of photophosphorylation leading to the death of plant cells.

Phosphinothricin acetyl transferase

The bacterial protein phosphinothricin acetyl transferase (PAT), encoded by the *bar* gene derived from *Streptomyces hygroscopicus*, is able to detoxify the herbicide. In *S. hygroscopicus*, the *bar* gene functions both as an integral part of the biosynthetic pathway for bialaphos in the bacteria[3], and as an enzyme which confers natural resistance (Kumada, 1988).

[3] Phosphinothricin was initially characterised as an antibiotic (bialaphos), which is produced naturally by the bacteria, but was later shown to be effective as a broad-spectrum herbicide. By acetylating the free amino group

When expressed in GM plants, PAT catalyses the conversion of L-PPT to N-acetyl-L-PPT, a chemical form of the herbicide that is unable to bind to and inactivate the plant GS. In LLRICE62, the 35S promoter used to express *bar* constitutively throughout the plant results in expression of the PAT protein in green tissues at sufficiently high levels to enable the plants to tolerate commercial applications of glufosinate-ammonium herbicides without detrimental effects.

The PAT enzyme is a homodimer of 183 amino acids with an apparent molecular weight of approximately 22 kDa; it is an acetyl transferase with enzyme specificity for both L-glufosinate (L-PPT) and demethylphosphinothricin (DMPT) in the acetylation reaction (Thompson *et al*., 1987). Both L-PPT and DMPT are inhibitors of glutamine synthetase. In the presence of acetyl-CoA, PAT catalyses the acetylation of the free amino group of L-PPT to N-acetyl-L-PPT, a herbicidally-inactive compound. The kinetics and substrate specificity of the PAT enzyme are well characterised; it has a high specificity for L-PPT and has been shown to have a very low affinity to related compounds and amino acids; even excess glutamate is unable to block the PPT-acetyltransferase reaction (Thompson *et al*., 1987).

The acetyltransferase activity is heat- and pH-dependent (Wehrmann *et al.*, 1996). PAT is active between temperatures of 25-55^{o}C (maximum activity at 40-45^{o}C). Complete thermoinactivation occurs at 60^{o}C (10 min) and above. The optimum pH for PAT activity is 8.5, but it is active over a broad pH range of 6 to 11.

4.2 Protein expression analysis

Studies submitted:

1. Phosphinothricin Acetyltransferase Content in Raw Agricultural Commodities of Event LLRICE62 Glufosinate Tolerant Rice, USA, 1998. Author: R.D. Shillito. Sponsor: Bayer CropScience Regulatory Affairs and Biotechnology, USA. Study Identification: BK98B102, completed May 2000.
2. Phosphinothricin Acetyltransferase Content in Processed Agricultural Commodities of Event LLRICE62 Glufosinate Tolerant Rice, USA, 1998. Author: R.D. Shillito. Sponsor: Bayer CropScience Regulatory Affairs and Biotechnology, USA. Study Identification: BK98B108, completed May 2000.

The expression levels of the PAT protein in LLRICE62 were evaluated in different plant tissues including grain, straw, stems, leaves and roots using a quantitative enzyme-linked immunosorbent assay (ELISA). This method is a sandwich immunoassay in which PAT specific polyclonal antibodies (goat) are used. The antiserum detects both degraded and intact PAT protein. A purified sample of *E. coli*-produced PAT was used as reference material for the positive control. The limit of detection (LOD) was determined by using the average standard curve and the concentration derived from the background optical density (OD) of the negative control samples. The LOD is the concentration corresponding to an OD value three standard deviations above the mean background OD.

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of L-PPT, the PAT enzyme prevents autotoxicity in the bacterial organisms and generates complete resistance towards high doses of L-PPT, bialaphos or the synthetically produced glufosinate-ammonium.

Rice grain and straw from field-grown plants at maturity and in leaves, stems and roots of late vegetative/panicle development stage were analysed for PAT using quantitative ELISA. The transgenic plot was treated twice with glufosinate ammonium herbicide at the rate of 0.45 pounds (active ingredient) per acre at approximately four and six weeks after planting. Plants were harvested 3 months later. Corresponding tissues from the non-transformed counterpart rice (Bengal) were used as negative controls. In the LLRICE62 samples, PAT protein constitutes 12.1 µg/g fresh weight (fw) of grain and 75.3 µg/g fresh weight of straw. These levels correspond to 0.02% and 0.32% of the crude protein respectively in these tissues. The levels of PAT protein evaluated from different seed lots in two successive years grown at the same location showed that the average PAT content in the grain is constant (see Table 2 below).

PAT levels in processed rice commodities

ELISA was used to evaluate the level of PAT protein in various processed rice fractions derived from LLRICE62, grown under the field conditions and herbicide regimen outlined above. Rough rice, hulls, brown rice, polished rice and parboiled brown rice were ground and extracts prepared. Further processing was not required for bran, rice flour and rice bran oil. Non-transgenic control rice fractions were prepared in the same manner. In this series of experiments, the limit of quantitation (LOQ) of the PAT immunoassay was found to be dependent on the matrix. The results are presented in Table 3, expressed as approximate percentage of total crude protein in the respective rice commodity. The processed fraction with the highest level of PAT protein is rice bran, with PAT constituting about 0.033% of the crude protein on a weight per weight basis.

Table 2: Levels of PAT protein in rice grain and straw from LLRICE62 at maturity, field-grown at same location in two successive years, as detected by ELISA; Percent of Crude Protein

Rice tissue	Average PAT content (µg/g fw ± SD)	Crude Protein in matrix (% w/w)	PAT Protein (% of crude protein)
Grain - year 1	**12.1 ± 0.6**	7.19	0.017
Straw - year 1	75.3 ± 4.4	2.38	0.316
Grain - year 2	**10.6 ± 1.3**	7.41	0.014

Table 3: PAT in Processed Agricultural Fractions of Transgenic Rice LLRICE62, as Detected by ELISA, as a Percentage of Crude Protein

Commodity	Crude Protein in matrix (% w/w)	PAT protein (% of crude protein)
Rough rice	7.06	0.0181
Rice hulls	2.40	0.0065
Brown rice	8.73	0.0152
Polished rice	7.79	0.0047
Rice bran	12.7	0.0331
Rice flour	9.04	0.0164
Rice bran oil	0	<LOQ
Parboiled brown rice	8.53	<LOQ

<LOQ – below the limit of quantitation

Studies submitted:

3. PAT Protein Content in Raw Agricultural Commodities of Event LLRICE62 Glufosinate Tolerant Rice, USA, 1999. Authors: R.D. Shillito & L.J. Macy. Sponsor: Bayer CropScience Regulatory Affairs and Biotechnology, USA. Study Identification: BK99B017, completed November 2002.

A further study reports the levels of PAT in the grain of transgenic rice event LLRICE62. Ten field trials, with planting dates from late March to mid May 1999, were conducted by the Agricultural Research Centre of Louisiana State University. At four sites, the transgenic rice was treated with glufosinate ammonium herbicide at the rate of 0.73 lb ai/A. At all ten sites, non-transgenic rice was also planted, however the experimental plots were randomized and therefore transgenic and non-transgenic rice were planted in adjacent plots. A plot combine was used to harvest the samples and an estimated 0.5% mixture of grain from adjacent plots was anticipated.

The average PAT protein concentration range was 9.5 – 11.1 µg/g fresh weight (mean 10.1 µg/g fresh weight) in the unsprayed transgenic rice grain. In the sprayed transgenic rice grain, the reported range was 6.8 – 10.9 µg/g fresh weight (mean 9.4 µg/g fresh weight). The average ratio of PAT protein to crude protein in the transgenic unsprayed samples (0.013%) and the transgenic sprayed samples (0.012%) was essentially the same. Although PAT protein was not present in the majority of control samples, very low levels of PAT were detected in some of the non-transgenic controls. PCR analysis confirmed that transgenic grain was present in detectable amounts in samples from control plots and that non-transgenic grain was also present in samples from transgenic plots. These results indicate that significant cross-contamination occurred as a result of the harvesting method used in the study. Notwithstanding the cross-contamination of samples, the results from this study correlate well with the levels of PAT detected in rice grain from previous trials conducted at different locations.

4.3 Characterisation of the novel protein in LLRICE62

The PAT protein is produced naturally by bacterial species commonly found in soil. The use of PAT enzymes to confer tolerance to glufosinate ammonium herbicides in other GM commodities has been assessed and a number of distinct lines are already approved. The potential toxicity and allergenicity of the PAT protein has been assessed by FSANZ on numerous occasions and no safety concerns were identified. Its use is approved in food derived from specific lines of soybean, corn, cotton and canola. New studies to characterise the PAT protein in LLRICE62 are relevant for this assessment.

Studies submitted:

1. Scott, A. Molecular Characterisation of Glufosinate-tolerant Rice Transformation Event LLRICE62. Sponsor: Bayer CropScience Regulatory Affairs and Biotechnology, USA, Report No. OS 24 v2, completed August 2006.
2. Currier, T.C. and Hendricks, K.. Structural and Functional Equivalence of PAT/bar protein produced in *Escherichia coli* and LLRICE62, *Oryza sativa*. Study ID: BK04Q015, completed October 2004.

Quantities of the PAT protein were produced in the laboratory as reference material by expression in *E. coli*.

This microbially-produced protein is used in toxicity and allergenicity studies and to establish that the PAT protein isolated from the leaves of LLRICE62 exhibits the same physical and biochemical properties as the reference material.

The coding region of the *bar* gene from *Streptomyces hygroscopicus* was modified for optimal gene expression in rice. As a result, there is one amino acid difference at the second N-terminal position of the PAT protein; a serine residue is present in rice compared with an aspartic acid residue in the *E. coli* form. Apart from this known difference, based on the nucleotide sequence of the coding regions, the protein produced in the rice is the same as the reference material produced in the laboratory.

Analytical tests such as SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis) and Western blots were used to identify and compare the plant- and microbially-produced PAT proteins. The amount of total extractable protein from the plant tissue samples was quantified using the Bradford method of analysis. The antibody preparation used in the Western blot analysis was a rabbit polyclonal antibody to the PAT protein produced by Bayer CropScience, and detection was via the use of alkaline phosphatase linked anti-rabbit antibody. The Western blot results show that the electrophoretic mobility and immunoreactivity of the PAT protein in the transformed rice were similar to the *E. coli*-produced PAT reference standard.

In a separate study, the novel protein was extracted from the leaves of LLRICE62 plants grown in the greenhouse and then affinity purified using goat antibodies. A number of different methods were used to demonstrate the equivalence of the microbial- and plant-derived PAT proteins. The results of these experiments are summarised as follows:

(1) N-terminal sequence – The N-terminal sequence for the PAT protein produced in LLRICE62 confirmed the expected N-terminal sequence based on the known change to the nucleotide sequence used for the rice transformation. The two PAT proteins differ at the N-terminal end only at the second amino acid residue (aspartic acid to serine in rice).

(3) Western blot analysis – the electrophoretic mobility and immunoreactivity of the PAT protein produced in LLRICE62 and *E. coli* were indistinguishable. Rabbit polyclonal antibodies to the PAT protein (Bayer CropScience) were used as the primary antibody, and the second antibody was a horseradish peroxidase linked anti-rabbit antibody.

(4) Enzyme activity – The functional activities of the plant-produced PAT protein and the *E. coli*-produced PAT reference standard were determined using a spectrophotometric assay. The enzyme assay demonstrated that both proteins were biologically active and thus the plant-produced protein is functionally equivalent to the *E. coli*-produced protein.

(5) Glycoprotein analysis – The PAT protein isolated from LLRICE62 plants and the *E. coli*-produced form were analysed for post-translational modification through covalently bound carbohydrate moieties. The procedure used a glycoprotein staining kit following SDS-PAGE. A set of glycoprotein molecular weight standards was included on the gel. This set of marker proteins forms an alternating ladder of glycosylated and non-glycosylated proteins. The presence of sugar residues on the proteins was tested using a commercial fluorescent glycoprotein detection kit. There was no detectable glycosylation of the plant-derived PAT protein using these methods.

(6) Molecular weight – The plant- and *E. coli*-produced PAT proteins co-migrated on SDS-PAGE. The apparent molecular weight of the two PAT proteins, estimated by comparison to molecular weight markers on the stained gel, was 21.2 kDa. This value compares favourably with the theoretical molecular mass of 20.6 kDa calculated from the amino acid sequence deduced from the DNA sequence of the native gene with a serine substitution at position 2.

A combination of N-terminal sequence analysis, SDS-PAGE and Western blots have confirmed the identity of the PAT protein produced in LLRICE62. The characterisation of the *E. coli*-produced PAT protein indicates it is equivalent to the plant-produced protein based on comparable electrophoretic mobility, enzyme activity, immunoreactivity and absence of detectable glycosylation. Based on the similarity of the results from the plant and microbial preparations, the *E. coli*-produced protein is chemically and functionally equivalent to the PAT protein expressed in LLRICE62.

4.4 Potential toxicity of novel proteins

Studies submitted:
Assessment of the toxicity and allergenicity of the PAT protein. Performing laboratory: Bayer CropScience, 355, rue Dostoievski, BP 153, 06903 Sophia Antipolis Cedex, France. Study Number:SA02218, completed in November, 2003.

The PAT protein in LLRICE62 is substantially similar to PAT proteins present in a number of GM food crops (e.g. canola and cotton), which have been assessed as safe for human consumption. Thus, approval of other glufosinate ammonium-tolerant food products expressing the PAT protein has provided a short history of safe use.

Data on the potential toxicity of PAT have been comprehensively assessed (see Final Assessment Reports for FSANZ Applications A372, A375, A380, A381, A386, A446, A481, A518, A525 and A543). The previous assessments considered history of exposure to the protein through the diet, bioinformatics analysis of the primary and secondary structure of the PAT protein to examine any similarities with known protein toxins, biochemical tests (heat stability, digestibility), and acute oral toxicity studies in mice. The previous assessments concluded that the PAT protein is not toxic and is safe for human consumption.

The Applicant has expanded the food safety assessment of the PAT protein for this commodity, to include both a review of published literature and experimental studies. The focus of the review is the *bar* gene product used in LLRICE62. However, the *pat* gene from *Streptomyces viridochromogenes* produces a similar PAT protein that has been used in corn and soybean to confer tolerance to glufosinate ammonium herbicides. Therefore, data used in these assessments is also relevant. As outlined in the previous section, a range of biochemical methods was used to establish that *E. coli* -produced PAT protein is equivalent to the protein produced by LLRICE62.

The complete amino acid sequence of the *bar*-encoded PAT protein is known. The total sequence was compared to known toxins listed in 7 large public databases.

As expected, the PAT protein only displayed high structural similarity to other non-toxic acetyltransferase proteins, which are common in nature. The overall homology search indicated no significant homology with any known protein toxins[4].

The acute oral toxicity of the PAT protein (at doses of 5000 mg/kg) has been studied in mice. The toxicity of PAT has also been studied following intravenous administration at two single dose levels of 1 and 10 mg/kg body weight. No adverse effects were observed in the animals after 15 days observation. At necropsy, body cavities were opened and organs examined *in situ* and removed. There were no pathological findings attributable to the treatment with the PAT protein[5]. Based on these results and previous studies, the PAT protein is considered non-toxic to mammals. There is now general consensus that the PAT protein is not toxic to either humans or other animals (OECD, 2002).

Potential toxicity of glufosinate ammonium metabolites

Two metabolic pathways operate in glufosinate-ammonium-tolerant plants to inactivate glufosinate-ammonium: N-acetylation of L-glufosinate producing N-acetyl-L-glufosinate (NAG) and the de-amination of glufosinate and its subsequent conversion to 3-[hydroxyl (methyl) phosphinoyl] propionic acid (MPP). NAG is generally the main metabolite that is formed. As these metabolites are a by-product resulting from the activity of an introduced enzyme, the safety of these compounds is considered in the assessment of LLRICE62.

NAG is considered non-toxic to plants, invertebrates, rodents and other mammals, including humans (OECD, 1999; Hoerlein, 1994). The committee of the Joint Meeting on Pesticide Residues (JMPR) has also reported that the metabolites resulting from the interaction of glufosinate-ammonium with PAT can be considered less toxic or equivalent to the toxicity of the parent compound (IPCS, 1999). An ADI (acceptable daily intake) level of 0 – 0.2 mg/kg body weight was established for glufosinate-ammonium, and its metabolites NAG and MPP (IPCS, 1999). Due to the low toxicity of glufosinate-ammonium and its metabolites, it was considered unnecessary to establish an acute reference dose.

4.5 Potential allergenicity of novel proteins

Almost all food allergens are proteins, however the vast majority of proteins in the diet are not allergens. The potential allergenicity of a novel protein can be evaluated using an integrated, step-wise, case-by-case approach relying on pieces of information used in combination, since no single criterion is sufficiently predictive of either allergenicity or non-allergenicity. The assessment focuses on whether:

(i) the source of the novel protein is a known allergen;
(ii) there is any significant sequence similarity of the novel protein with that of known allergens; and
(iii) the physical properties of the novel protein, including susceptibility to heat and simulated digestive fluids, indicate resistance to normal proteolytic degradation.

[4] Herouet, C. (2002). Phosphinothricin-Acetyl-Transferase(PAT)- *bar* gene product. Overall amino acid sequence homology search with known toxins and allergens. Aventis CropScience # C024579
[5] Kennel, P. (2002). Aventis CropScience unpublished study # C025883

When the findings indicate the necessity for further testing (e.g. if the source of the novel protein is a food known to be allergenic), additional *in vitro* and *in vivo* immunological testing on the protein can be conducted. Applying such criteria systematically provides reasonable evidence on the potential of the novel protein to be allergenic.

Previous assessment of the PAT protein for potential allergenicity

A number of studies to examine the potential allergenicity of the PAT protein have been submitted previously for safety assessment[6]. In addition to the broad bioinformatics studies described above, the established databases were analysed in finer detail for the existence of shared linear epitopes (or putative immunoreactive sequences) between the PAT protein and known allergens. This approach focused on any short sequences of amino acids in common with known allergens (eight linearly contiguous identical amino acids, which is the minimum peptide length for a T-cell binding epitope). No sequence similarities with an allergenic epitope were observed. Information on epitopes created by secondary or tertiary protein structure (conformational epitopes) is not available. In addition, an *in silico* search using specific consensus sequences of potential glycosylation sites, often found in allergenic proteins, revealed no N- and O-glycosylation motifs in the PAT protein. Biochemical analysis described in Section 4.3 above did not reveal post-translational glycosylation of the PAT protein produced in LLRICE62.

Heat stability

The PAT protein is detectable by SDS-PAGE after treatment at temperatures up to 90°C for 10 minutes. However, PAT enzyme activity is inhibited at temperatures above 40-45°C for 15 minutes, and complete thermoinactivation occurs after 10 minutes at 60°C or above. The stability of food allergens to high temperature processing (heat denaturation) places importance on the bioinformatic analysis to identify any potential linear epitopes in the novel protein.

In vitro digestibility

Typically, food proteins that are allergenic tend to be stable to enzymes such as pepsin and the acidic conditions of the digestive system, allowing exposure to the intestinal mucosa where absorption and sensitisation can occur leading to an allergic response (Metcalfe *et al*., 1996; Astwood *et al*., 1996; Kimber *et al*., 1999). For example, several allergens are known to be stable for up to 24 hours under simulated digestive conditions. Novel proteins are therefore investigated for their digestibility in simulated digestion models as part of the assessment of potential allergenicity.

A number of *in vitro* digestibility experiments have demonstrated that the PAT protein expressed in LLRICE62 is readily digested under simulated gastric and intestinal conditions.

[6] Studies by Aventis CropScience, 355, rue Dostoievski, BP 153, F-06903 Sophia Antipolis Cedex, France:
Herouet, C. (2002) Epitope homology and glycosylation searches. Unpublished Study # SA02199.
Esdaile, D.J. (2002) *In Vitro* digestibility study in simulated gastric fluid. Unpublished Study # SA02173.
Esdaile, D.J. (2002) *In Vitro* digestibility study in simulated intestinal fluid. Unpublished Study # SA02174.

Solutions of PAT were incubated with simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) for different periods of time and subsequently analysed by SDS-PAGE and Western blot analysis. No residual protein was visible after 30 seconds incubation with SGF, in the presence of pepsin, at pH 2.
Similarly, the PAT protein was digested within seconds when incubated with SIF and pancreatin, at pH 7.5. In the absence of the proteases pepsin and pancreatin, the PAT protein remained substantially intact.

Another study demonstrated that the PAT protein was no longer detectable by a silver-stained SDS-PAGE analysis after a brief incubation in simulated human gastric fluid (Wehrmann *et al*., 1996). This study also confirmed that PAT was not degraded when pepsin was omitted from the reaction mixture.

4.6 Conclusion

The PAT protein is constitutively expressed in LLRICE62 and was detected by quantitative ELISA in straw, stems, leaves and roots and at very low levels in the unprocessed grain. When grown under normal field conditions, PAT constitutes approximately 12.1 µg/g fresh weight in grain which corresponds to about 0.02% of the crude protein. In commodity fractions processed from the grain, PAT levels are proportionally highest in rice bran where it constitutes about 0.03% of the crude protein. Plant proteins including PAT were not present at all in rice bran oil.

A number of studies to investigate the potential toxicity and allergenicity of the PAT protein have been evaluated. The PAT protein produced in LLRICE62 is chemically and functionally equivalent to *E. coli*-produced PAT protein based on comparable electrophoretic mobility, enzyme activity, immunoreactivity and absence of detectable glycosylation. Previous assessments of acute toxicity studies on the microbially-produced PAT protein are therefore relevant to the safety assessment of LLRICE62; no toxicity was observed in mice at oral doses up to 5000 mg/kg and intravenous doses up to 10 mg/kg. The PAT protein does not exhibit sequence similarities with known toxins or allergens, and demonstrates digestive lability in conditions that mimic human digestion. The protein demonstrates some heat stability however, given the combined evidence from other studies indicating that it is not toxic and unlikely to be allergenic, this result does not by itself raise a safety concern.

5. COMPOSITIONAL ANALYSES

A comparison of similarities and differences in composition between a GM plant and its conventional counterpart aids in the identification of potential safety and nutritional issues and is considered one of the most important elements of the safety assessment of GM foods (WHO, 2000). When determining similarities and differences in composition between a GM plant and its conventional counterpart, the critical components measured are determined by identifying key nutrients, key toxicants and anti-nutrients for the food source in question (FAO, 1996). The key nutrients and toxicants/anti-nutrients are those components in a particular food that have a substantial impact in the overall diet. These can be major constituents (e.g., fats, proteins, carbohydrates) or minor constituents (e.g., minerals, vitamins). Key toxicants are those toxicologically significant compounds known to be natural constituents of the plant and whose potency and level may be significant to health (e.g., increased levels of solanine in potatoes). The key components of rice include the proximates, minerals, vitamins, fatty acids, amino acids and phytic acid (OECD 2004).

Levels of key nutrients and other constituents

Studies submitted:
Composition of Processed Fractions of Event LLRICE62 Glufosinate Tolerant Rice, USA, 1998, R.D. Shillito. Aventis CropScience Study Id. BK98B110. Study completed in August 2000.

Multiple analytical studies were conducted to determine the composition of processed agricultural fractions of GM rice event LLRICE62 and the non-transformed parental line (var. Bengal), as outlined in Table 4. The whole grain was supplied from rice grown in 1998 (May to September) in a primary rice growing region of the USA in EPA Region IV, at the Louisiana State University Agricultural Center, Rice Research Station, in Louisiana.

The rice was grown under conditions typical of agricultural production practices. There was one transgenic and one non-transgenic plot at the test site. The transgenic crop was treated twice with glufosinate-ammonium at a rate of 500g per hectare per application. The whole grain was processed by the Food Protein and Development Center, Texas A&M University. Samples of whole rice grain were removed and frozen for analysis before processing.

Mature rice grain is harvested as a covered grain (known as rough rice or paddy rice). For the compositional studies the commodities produced for analysis were: brown rice, polished rice, hulls, bran, rice, flour, bran oil (crude), and parboiled brown rice (see Figure 1). The processed commodities were shipped to (i) Woodson-Tenent Laboratories and Ralston Analytical Laboratories for compositional analysis, (ii) AgrEvo Research Center for determination of rice allergenic protein, and (iii) Riceland Foods and USDA Western Regional Research Center for analysis of the bran oil. Samples of brown rice were shipped to the University of Arkansas for determination of the rice storage proteins.

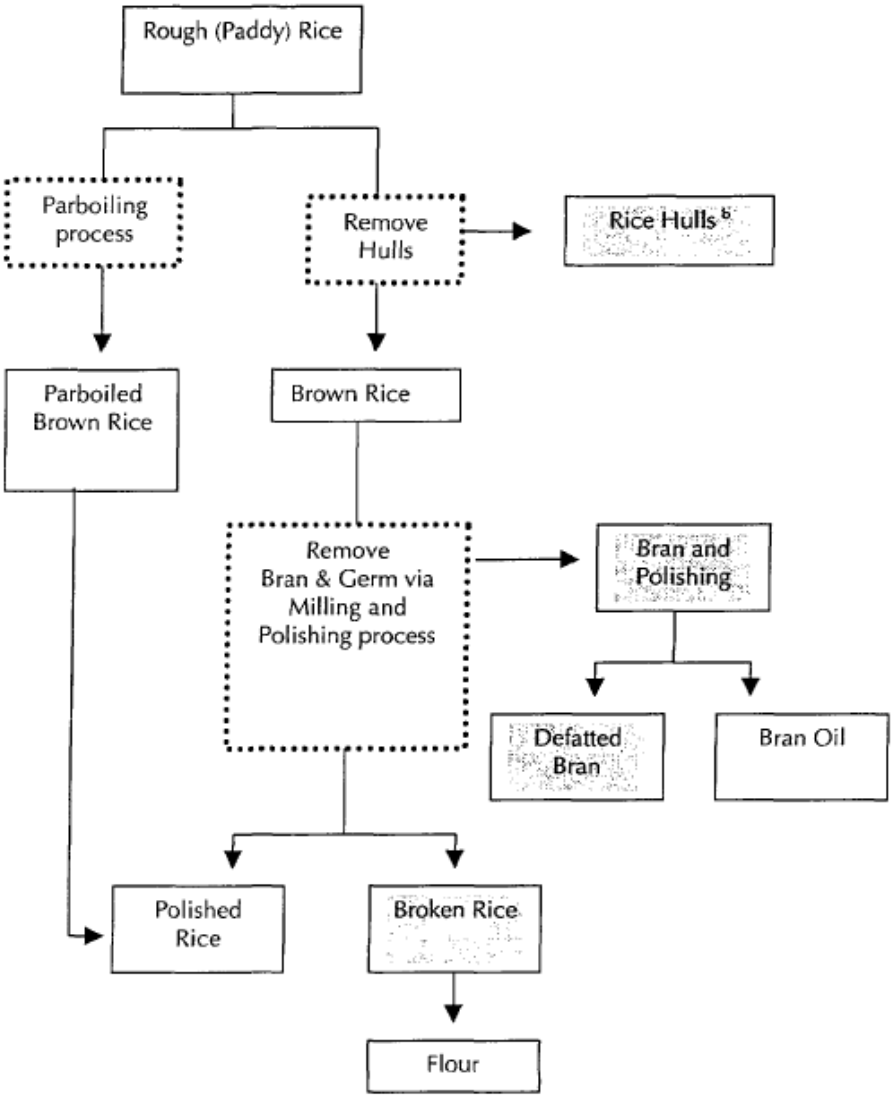

Figure 1: Rice Processing (outlined in broken lines) and Products (outlined in solid lines) (Amann, 1998)

Results

The results of all analyses of commodities listed in Table 4 do not show any significant differences between LLRICE62 and the non-GM parental line, however the field trail was limited in scope. The data from this study have been combined with data from 3 other separate studies: another study from a trial conducted in the 1998 growing season, and two further studies in 1999 on field trials at different locations. The results from all studies have been compiled into a larger report (see following section) for detailed statistical analysis.

Table 4: Analyses Performed on Processed Agricultural Commodities of GM Event LLRICE62 and Non-GM Counterpart

Sample	Analysis performed
Grain (Rough Rice or Paddy Rice)	total protein, total fat, moisture, carbohydrate calculation, ash, acid detergent fiber, neutral detergent fiber, total dietary fiber, insoluble and soluble dietary fiber, amino acids including tryptophan, fatty acids, phosphorous, iron, calcium, vitamins*, trypsin inhibitor, phytic acid and lectin.
Hulls	total protein, total fat, moisture, carbohydrate calculation, ash, acid detergent fiber, neutral detergent fiber, total dietary fiber, and insoluble and soluble dietary fiber
Brown rice	total protein, total fat, moisture, carbohydrate calculation, ash, amino acids including tryptophan, phosphorous, iron, calcium, vitamins, rice allergenic protein, albumin, globulin, glutelin and prolamin
Parboiled brown rice	total protein, total fat, moisture, carbohydrate calculation, ash, amino acids including tryptophan, phosphorous, iron, calcium and vitamins*
Polished rice	total protein, total fat, moisture, carbohydrate calculation, ash, amino acids including tryptophan, fatty acids, iron, calcium, vitamins*, trypsin inhibitor, phytic acid and lectin
Flour (dry milled)	total protein, total fat, moisture, carbohydrate calculation, ash, amino acids including tryptophan, fatty acids, iron, calcium and vitamins*.
Bran	total protein, total fat, moisture, carbohydrate calculation, ash, acid detergent fiber, neutral detergent fiber, total dietary fiber, insoluble and soluble dietary fiber, amino acids, fatty acids, phosphorous, vitamins*, trypsin inhibitor, phytic acid and lectin
Bran oil	fatty acids, tocopherols, tocotrienols, oryzanol

* Vitamins measured were: niacin, thiamine (B1), Riboflavin (B2), Pantothenic Acid and Vitamins A and E.

Studies submitted:
Nutritional Impact Assessment Report on Glufosinate Tolerant Rice Transformant LLRICE62, R. Oberdorfer. Aventis CropScience, Frankfurt, Germany. Report No. N1 01 EUR 01, completed in September 2001.

Evaluations of rice commodities from four separate studies were used to provide a detailed compositional analysis of LLRICE62 over multiple growing seasons and in different environments. Multiple field trials were conducted on LLRICE62 (with and without herbicide treatment), the medium-grain parent line variety Bengal (the conventional counterpart), and other varieties of rice. Samples were generated over two years (1998 and 1999) at 14 different trial sites to compensate for any environmental effects (such as variable soil fertility or water availability) at individual sites. In four of the trials, LLRICE62 plots were treated twice with 818g per hectare of glufosinate ammonium herbicide; remaining trials involved application rates of 500g per hectare (as noted above). Treatment plots were planted in replicate and replicate samples were harvested from each treatment plot.

Since every downstream product from the rice grain is used for human food or animal feed, all were included in the analyses which generated a large data set. Parameters measured include: proximates, amino acids, fatty acids, micronutrients (such as vitamins and minerals), and three anti-nutrients of importance for rice and rice products (phytic acid, trypsin-inhibitors and lectins). The primary data from each set of analyses for each trial site were provided. This large data set was subjected to detailed statistical evaluation, and the pooled results for rice grain obtained from the four studies are given in Tables 5, 6, 7 and 8.

The standard range used in the comparison was compiled from a large number of published references reporting the composition of rice grain, including cereal reference texts and technical publications. The Applicant noted however that no information was available in these texts on the commercial rice varieties, the analytical methods, or the statistical analyses used to generate the values, and therefore a direct comparison with LLRICE62 and its medium-grain parental variety may not be applicable. Notwithstanding limited information, the reference range provides a broad base for comparing compositional parameters in LLRICE62, the conventional parental line, and other commercial varieties of rice with a safe history of consumption.

Results from the combined sites analysis

The results of the detailed statistical analysis on the composition of LLRICE62 and the non-transgenic counterpart have been published in the Journal of Agricultural and Food Chemistry (Oberdoerfer *et al*., 2005).

The data from the combined site comparisons from the 1998 and 1999 field seasons were subjected to statistical analysis to calculate variance (ANOVA). Statistically significant differences were determined at the 5% level of significance ($p<0.05$). SAS® software was used to generate all summary statistics and perform all analyses. The Applicant used a coefficient of variance of ±20% of the reference mean as the range corresponding to natural biological variation. In an analysis of this magnitude, a small percentage (approximately 5%) of statistically significant differences is expected to occur due to chance alone.

Table 5: Proximate Analysis in Grain of Rice Event LLRICE62 and the Non-Transgenic Counterpart (combined data from 4 studies)

Parameter	Percentage dry matter			
	Non-GM parental line	**LLRICE 62 Untreated**	**LLRICE 62 Treated**	**Standard Values [a]**
Moisture	10.99	10.42	12.93	11.0-13.7
Crude Fat	2.57	2.61	2.62	1.80-2.70
Crude Protein	8.10	8.41	8.31	6.70-8.90
Ash	4.55	4.47	4.69	3.40-6.00
Crude Fibre	10.36	10.61	10.45	8.40-12.10
ADF	14.68	14.31	14.13	NF
NDF	18.10	19.44	17.93	16.40 [c]
TDF	18.84	19.41	18.42	19.10
Total carbohydrates [b]	84.78	84.51	84.38	83.00-87.80

NF no data found
a Standard range compiled from reference material
b Total carbohydrates calculated as 100% - (crude protein %dm + crude fat %dm + ash %dm)
c Single value obtained from reference (Ensminger, 1990)

Table 6: Amino Acids in Grain of Rice Event LLRICE 62 and the Non-Transgenic Counterpart (combined data from 4 studies)

Amino Acid	Percentage dry matter			
	Non-GM parental line	**LLRICE 62 Untreated**	**LLRICE 62 Treated**	**Standard Values [a]**
Alanine	0.41	0.42	0.42	0.47
Arginine	0.55	0.55	0.55	0.52-0.80
Asparagine	0.73	0.74	0.73	0.81
Cysteine	0.18	0.19	0.18	0.09-0.14
Glutamic Acid	1.25	1.30	1.26	1.59
Glycine	0.36	0.36	0.36	0.39-0.69
Histidine	0.19	0.20	0.21	0.10-0.20
Isoleucine	0.28	0.29	0.29	0.30-0.43
Leucine	0.58	0.59	0.59	0.60-0.68
Lysine	0.29	0.29	0.29	0.28-0.34
Methionine	0.19	0.20	0.19	0.15-0.20
Phenylalanine	0.37	0.38	0.37	0.34-0.42
Proline	0.34	0.35	0.35	0.37
Serine	0.38	0.38	0.38	0.41-0.56
Threonine	0.28	0.28	0.28	0.26-0.35
Tryptophan	0.10	0.10	0.10	0.10-0.14
Tyrosine	0.13	0.12	0.13	0.26-0.71
Valine	0.41	0.43	0.42	0.44-0.58

a Standard range compiled from reference material

Table 7: Minerals, Vitamins and Phytic Acid in Grain of Rice Event LLRICE62 and the Non-Transgenic Counterpart (combined data from 4 studies)

Parameter	As dry matter			
	Non-GM parental line	**LLRICE 62 Untreated**	**LLRICE 62 Treated**	**Standard Values** [a]
Calcium %	0.022	0.027	0.028	0.02-0.07
Phosphorus %	0.268	0.278	0.286	0.24-0.36
Potassium %	0.286	0.297	0.294	0.18-0.53
Iron mg/kg	35.85	50.52	41.44	16.2-57.0
Niacin mg/kg	48.76	49.86	54.73	14.6-65.0
Pantothenic acid mg/kg	9.10	10.52	11.10	4.0-12.4
Vitamin B1 mg/kg	5.28	5.89	5.96	1.4-3.8
Vitamin B2 mg/kg	1.11	1.10	1.12	0.4-1.3
Vitamin E IU/kg	17.30	20.76	19.70	6.7-34.7
Phytic acid %	0.83	0.86	0.81	0.72-1.20

a Standard values compiled from reference material

Table 8: Fatty Acids in Grain of Rice Event LLRICE 62 and the Non-Transgenic Counterpart (combined data from 4 studies)

Fatty Acid	Percentage			
	Non-GM parental line	**LLRICE 62 Untreated**	**LLRICE 62 Treated**	**Standard Values** [a]
C14:0 Myristic Acid	0.38	0.36	0.33	1.0-1.5
C16:0 Palmitic Acid	15.38	15.18	15.13	17.6-28.0
C16:1 Palmitoleic Acid	0.32	0.32	0.34	0.5-6.0
C18:0 Stearic Acid	1.92	1.96	1.97	2.0
C18:1 Oleic Acid	39.88	40.33	40.24	35.0-47.6
C18:2 Linoleic Acid	37.48	37.08	37.34	34.0-39.0
C18:3 Linolenic Acid	1.08	1.06	1.11	0.8-3.0
C20:0 Arachidic Acid	0.73	0.74	0.74	NF
C20:1 Gadoleic Acid	0.61	0.58	0.56	NF
C22:0 Behenic Acid	0.54	0.56	0.56	NF
C22:1 Erucic Acid [b]	0.18	0.27	0.14	NF
C24:0 Lignoceric Acid	1.11	1.14	1.13	NF
C24:1 Nervonic Acid	0.14	0.14	0.15	NF

a Standard values compiled from reference material

b Only those sites in which more than one third of the values were measurable were considered.

Proximate analysis

Results from the proximate analyses conducted on grain samples derived from LLRICE62 plants and the non-GM control indicated no significant differences in crude protein, crude fat, ash, moisture, total dietary fibre and total carbohydrate. At some individual sites, there were differences in crude fibre, acid detergent fibre (ADF) and neutral detergent fibre (NDF), but these were found to be more site dependent than related to the treatment group; that is, the observed differences could not be correlated with the genetic modification.

Amino acids

In the combined amino acid analyses, there were largely no differences between the LLRICE62 samples and the non-GM counterpart. Differences in tyrosine were observed across a number of individual trial sites and ranged broadly from -29.8% to +47.0% of the control mean, however no pattern of difference associated with the genetic modification was observed. Similarly, observed differences in the tryptophan results across some sites was small and confined to the comparison between the non-GM and unsprayed GM plant; there was no difference in tryptophan levels between the sprayed GM rice and the non-GM control.

Fatty acids

There were no significant differences in the following fatty acids in LLRICE62 samples (sprayed and unsprayed) compared with the non-GM control: C14:0, C16:0, C18:0, C18:1, C18:2, C18:3, C20:0, C20:1 and C24:0. At a number of individual sites, C16:1 (palmitoleic acid) and C22:0 (behenic acid) levels in the GM grain were outside of the accepted 20% tolerance range however values occurred above and below the range and were not consistent across sites. Overall, in the combined results, there were no significant differences in the levels of palmitoleic acid and behenic acid across the three treatment groups (i.e. non-GM control, GM rice unsprayed and GM rice sprayed). The levels of C22:1 (erucic acid) in the rice grain were close to the limit of quantitation and showed some degree of variation; this observation is not considered to be associated with the genetic modification.

Nutrients

The analysis of minerals and vitamins found no significant differences between LLRICE62 samples and the non-GM control for the majority of parameters measured, with the exception of vitamin E and iron. In the vitamin E analysis, the study authors noted inherent variability in the analytical method used for the comparison; even replicate samples fluctuated either side of the reference range. The combined sites analysis showed a statistically significant increase in the levels of vitamin E in the GM rice samples compared to the non-GM control. The vitamin E level in non-GM control grain was 39% lower than unsprayed LLRICE62, but 55% lower than sprayed LLRICE62. When compared to the literature values, the absolute values for the GM rice, sprayed and unsprayed, were within the range reported in the literature for other commercial rice varieties currently on the market (see Table 9 for combined results).

Table 9: Nutrients in LLRICE62 Grain, sprayed and unsprayed, and the Non-Transgenic Counterpart (combined analysis)

Nutrient	Non-GM control	LLRICE62 (unsprayed)	LLRICE62 (sprayed)	Literature range
Vitamin E IU/kg dm	14.0-25.6	16.3-26.5	16.7-23.7	6.7-34.7
Iron mg/kg dm	19.7-67.0	41.5-65.4	29.0-51.0	16.2-57.0
Vitamin B1 mg/kg dm	2.9-6.2	5.1-7.0	5.2-7.0	1.4-3.8

The results for the nutrient iron also suggested higher levels in the transgenic rice (unsprayed) compared to the non-transgenic control, however this trend was not evident in the comparison between the sprayed transgenic rice and the control. The difference between the two transgenic groups (i.e. unsprayed and sprayed) was statistically significant. Overall, both the non-transgenic control as well as the transgenic samples ranged outside of the values reported in the literature for this nutrient (Table 9). These observations were not considered to be associated with the genetic modification but rather were most likely due to other variables.

For all treatment groups in these studies (i.e. non-transgenic control, unsprayed transgenic LLRICE62 and sprayed transgenic rice), the vitamin B1 levels in rough rice exceeded the reported literature range by a significant margin (Table 9). As for other nutrients that showed similar deviations from the literature range, the results do not reflect differences attributed to the genetic modification and are not considered to represent biologically meaningful differences between the transgenic line and its conventional counterpart.

Anti-nutrients

Trypsin inhibitor and haemagglutinin were not detected in any of the rice grain samples. The results for phytic acid showed less than 10% variance in all samples at all sites for all treatment groups, which represents no significant difference between the transgenic rough rice (sprayed or unsprayed) and the non-transgenic control.

Compositional analysis of rice flour

The composition of flour milled from LLRICE62 rice grain and the non-transgenic parental line was evaluated and the results are presented in Table 10. Proximates, amino acids, minerals and vitamins were measured and compared to a standard literature range sourced from various published references.

There were no significant differences between the non-transgenic rice and LLRICE62 in a range of parameters relevant to the composition of rice flour. It is noted that both groups exhibited higher protein, fat and ash levels and lower carbohydrate levels compared to the literature range. As could be expected from higher amounts of protein, the levels of almost all amino acids are correspondingly higher than the literature range. The vitamin and mineral content of flour derived from LLRICE62 grain is comparable to that present in grain from the non-transgenic counterpart, and it is noted again that both groups deviate significantly from the literature range.

Table 10: Compositional Analyses of Rice Flour from LLRICE62 and the Conventional Counterpart

Proximates	Percentage dry matter		
	Non-GM control	**LLRICE62**	**Reference range**
Crude Fat	2.51	2.47	0.7-1.6
Crude Protein	10.34	10.43	6.8-7.6
Ash	1.56	1.57	0.6-0.7
Total carbohydrates [a]	85.6	85.54	91
Amino Acids	Percentage dry matter		
Alanine	0.51	0.52	0.38-0.50
Arginine	0.79	0.80	0.58-0.66
Asparagine	0.97	0.96	0.62-0.77
Cysteine	0.22	0.24	0.10-0.12
Glutamine	1.71	1.79	1.24-1.31
Glycine	0.44	0.45	0.30-0.39
Histidine	0.29	0.30	0.17-0.20
Isoleucine	0.38	0.39	0.28-0.38
Leucine	0.75	0.77	0.55-0.71
Lysine	0.35	0.35	0.24-0.32
Methionine	0.25	0.29	0.16-0.22
Phenylalanine	0.47	0.50	0.36-0.45
Proline	0.43	0.46	0.32-0.38
Serine	0.50	0.51	0.35-0.47
Threonine	0.36	0.36	0.24-0.27
Tryptophan	0.14	0.14	0.08-0.11
Tyrosine	0.22	0.23	0.32-0.36
Valine	0.55	0.55	0.40-0.57
Minerals and Vitamins	Dry matter		
Calcium %	<0.011	<0.011	0.008-0.011
Iron (ppm)	16.17	13.84	4.0-4.6
Niacin (ppm)	54.49	50.86	16-29
Vitamin B1 (ppm)	5.27	5.80	0.69-1.57
Vitamin B2 (ppm)	1.07	0.88	0.24-0.34

a Total carbohydrates calculated as 100% - (crude protein %dm + crude fat %dm + ash %dm)

5.3 Conclusion

In a study of this magnitude, a small percentage (approximately 5%) of statistically significant differences is expected to occur due to chance alone. Differences occurring in one of the field sites only which are not repeated at other sites, are not indicative of a pattern of change that could be attributed to the genetic changes and are more likely to be random occurrences. In this comparative study, changes in the levels of some analytes are in this category. Consequently, these differences, although statistically significant for the individual site, are not considered to be biologically meaningful.

Detailed comparative analyses of proximates, amino acids, fatty acids, minerals and vitamins and anti-nutrients relevant to rice do not indicate any compositional differences of biological significance in the grain derived from LLRICE62 compared to the non-GM parental line when grown in conditions typical of commercial rice production. Although small differences in the levels of tryptophan and tyrosine were observed for LLRICE62 and the non-GM parent at some individual sites, this was likely to be due to localised variables and the absolute levels were well within the range expected for these amino acids for conventionally produced commercial rice varieties. Hence, these differences are unlikely to be biologically meaningful. The levels of other components of LLRICE62 that are statistically significantly different from the non-GM control population show a broad natural variation and do not raise any nutritional concerns. Overall, rice grain derived from LLRICE62 can be considered equivalent in composition to grain from conventionally produced rice varieties.

6. NUTRITIONAL IMPACT

Establishing that a GM food is safe for human consumption is generally achieved through an understanding of the genetic modification and its direct consequences in the plant, together with an extensive compositional analysis of the food.

To date, all approved GM plants with modified agronomic production traits (e.g. herbicide tolerance) have been shown to be compositionally equivalent to their conventional counterparts. Feeding studies in animals using feeds derived from compositionally equivalent GM plants have also shown equivalent nutritional performance to that observed with non-GM feed. Thus the evidence to date is that where GM varieties have been shown to be compositionally equivalent to conventional varieties, feeding studies using target livestock species contribute minimally to a safety assessment.

This approach would not apply to plants engineered with the intention of significantly changing their composition or nutrient bioavailability and thus their nutritional characteristics. In these cases, it is recognised that suitable comparators may not be available for a nutritional assessment based on compositional analysis. In such cases, feeding trials with one or more target species may be useful to demonstrate wholesomeness in appropriate test animals.

In this case, LLRICE62 is the result of a simple genetic modification to confer herbicide tolerance with no intention to significantly alter nutritional parameters in the food. In addition, extensive compositional analyses have been undertaken to demonstrate the nutritional adequacy of LLRICE62 and these indicate it is equivalent in composition to grain from conventionally produced rice varieties. The Applicant has however submitted two feeding trials comparing the nutritional performance of LLRICE62 with non-GM varieties as supporting information. These studies are summarised below.

Feeding study in swine

LLRICE62 rice was compared with a near-isogenic conventional medium-grain cultivar and a commercially milled long-grain rice in the diet for growing–finishing pigs. The results of the study have been published in the Journal of Animal Science (Cromwell *et al*., 2005).

One of four fortified rice-soybean meal diets was fed to growing-finishing pigs (n=96) from approximately 25 kg individual bodyweight until slaughter at approximately 106 kg individual bodyweight. The four test diets were: grain from LLRICE62 fields treated with glufosinate ammonium herbicide, untreated LLRICE62 grain, a near-isogenic conventional brown rice, and commercially milled long-grain rice. Diets were fortified with decreasing amounts of lysine at the growing, early-finishing and late-finishing phases respectively. The percentage of rice in the four diets was constant during each phase: 72.8, 80.0 then 85.8% for the growing, early-finishing, and late-finishing phases respectively. At the end of the 98 day experiment, bodyweight gain, feed intake and feed:gain ratio were evaluated as well as carcass data. The results showed similar growth performances in all treatment groups; gilts grew slower ($P<0.05$) and were leaner ($P<0.05$) than barrows. Carcass traits, adjusted for final bodyweight, did not differ between treatment groups. There was also no difference in response to the type of rice in the diet between barrows and gilts, with no evidence of a diet-gender interaction. The conclusion from the study was that LLRICE62 was found to be similar in nutritional value to conventional rice for growing-finishing pigs.

Feeding study in broiler chickens

To test the nutritional equivalence of LLRICE62 in another species, 120 male broiler chickens (one day old) were divided into two groups of 60 animals: one group received a diet containing 30% transgenic rice event LLRICE62 while the other group received a diet containing 30% rice from the conventional counterpart (near isogenic line). The raw rice grain was cleaned, sieved and milled on a hammer mill before mixing through the feed. Throughout the 42 day experiment, the diets were fed *ad libitum* through a feed hopper. The behaviour and physical condition of the birds were observed twice daily. Individual body weights were measured at day 7, 14, 21, 26, 35 and at the end of the study. Feed conversion efficiency was calculated from regular measurements of body weight and feed intake. Carcass parameters of interest in this study included carcass weight, breast muscle and abdominal fat weights. In addition, clinical signs and macroscopic findings were recorded by a pathologist.

No significant differences ($P>0.05$) were found in feed intake, feed conversion efficiency, weight gain or slaughter quality parameters between birds receiving the transgenic or non-transgenic diet groups. Two birds died during the experiment: one on Day 3 due to a disorder of the yolk sac and bleeding, and the second on Day 37 as a result of ascitis. At the end of the study, three different abnormalities were found at *post mortem* in about half of the birds from both the non-transgenic and transgenic diet groups. The observed mortality rate and the macroscopic findings are considered typical of broiler production. The conclusion from the study was that a diet containing transgenic rice event LLRICE62 was nutritionally equivalent to a diet containing conventional non-transgenic rice in broiler poultry.

REFERENCES

Amann, M.M. (1999) Identification of nutrients and antinutrients to analyse in cotton-derived foods for human and animal consumption. Environ, Arlington, Virginia, USA.

Astwood, J.D., Leach, J.N. and Fuchs, R.L. (1996) Stability of food allergens to digestion *in vitro*. *Nat.Biotechnol.* 14(10):1269-1273.

Bradbury, J.F. (1986) Guide to plant pathogenic bacteria. The Cambridge News Ltd Aberystwyth, 190-197.

FAO (1996) *Biotechnology and food safety. A report of a Joint FAO/WHO Consultation. Food and Agriculture Organisation, Food and Nutrition Paper 61, Food and Agriculture Organization of the United Nations, Rome.*

IPCS (International Programme on Chemical Safety) 1999. Toxicological evaluations: Glufosinate ammonium (addendum). http://www.inchem.org/documents/jmpr/jmprmono/v99pr06.htm

Kumada, Y., Anzai, H., Takano, E., Murakami, T., Hara, O., Itoh, R., Imai, S., Satoh, A. and Nagaoka, K. (1988) The bialaphos resistance gene (*bar*) plays a role in both self-defence and bialaphos biosynthesis in *Streptomyces hygroscopicus*. *J. Antibiotics* 41:1838-1845.

Kimber, I., Kerkvliet, N.I., Taylor, S.L., Astwood, J.D., Sarlo, K. and Dearman, R.J. (1999) Toxicology of protein allergenicity: prediction and characterization. *Toxicol.Sci* 48(2):157-162.

Kutzner, H.J. (1981) The family Streptomycetaceae. In: The Prokaryotes: a handbook on habitats, isolation and identification of bacteria. Starr, M.P., Stop, H., Truper, H.G., Ballows, A. and Schlegel, H.G. (Eds) Springer Verlag, Berlin, London.

Metcalfe, D.D., Astwood, J.D., Townsend, R., Sampson, H.A., Taylor, S.L. and Fuchs, R.L. (1996) Assessment of the allergenic potential of foods derived from genetically engineered crop plants. *Crit Rev Food Sci Nutr* 36 Suppl:S165-S186.

Murakami, T., Anzai, H., Imai, S., Sathah, A., Nagaoka, K. and Thompson, C.J. (1986). The bialaphos biosynthetic genes of *Streptomyces hygroscopicus*: Molecular cloning and characterisation of the gene cluster. *Molec. Gen. Genet*. 205: 42-50.

Oberdoerfer, R.B., Shillito, R.D., de Beuckeleer, M. and Mitten, D.H. (2005) Rice (*Oryza sativa* L.) Containing the *bar* Gene is Compositionally Equivalent to the Nontransgenic Counterpart. *J. Agric. Food Chem.* 53:1457-1465.

OECD (1999) *ConsensusDocument on the Biology of Oryza Sativa (Rice)*. Series on Harmonisation of Regulatory Oversight in Biotechnology No. 14, Organisation for Economic Co-operation and Development, Paris, France.

OECD (2002) Series on harmonisation of Regulatory Oversight in Biotechnology, No. 25. ModuleII: Phosphinothricin. Organisation for Economic Co-operation and Development, Paris, France.

OECD (2004) Consensus Document on Compositional Considerations for New Varieties of Rice (*Oryza sativa*): Key Food and Feed Nutrients and Anti-nutrients. Environmental Directorate, Organisation for Economic Co-operation and Development, Paris, France.

Southern, E.M. (1975) Detection of specific sequences among DNA fragments separated by gel electrophoresis. *Journal of Molecular Biology* 98(3):503-517.

Thompson, C.J., Movva Rao, N., Tizard, R., Crameri, R. Davies, J., Lauwereys, M. and Botterman, J. (1987) Characterisation of the herbicide resistance gene *bar* from *Streptomyces hygroscopicus*. The EMBO Journal 6: 2519-2523.

Walker, K.A. and Sato, S.J. (1981) Morphogenesis in callus tissue of *Medicago sativa*: the role of ammonium ion in somatic embryogenesis. *Plant cell, tissue and organ culture* 1(2):109-121.

Wehrmann, A., Van Vliet, A., Opsomer, C., Botterman, J. and Schultz, A. (1996) The similarities of *bar* and *pat* gene products make them equally applicable for plant engineers. *Nature Biotechnology* 14:1274-1278.

WHO (2000) *Safety aspects of genetically modified foods of plant origin. Report of a Joint FAO/WHO Expert Consultation, World Health Organization, Geneva.*

Yoo, S.Y., Bomblies, K., Yoo, S.K., Yang, J.W., Choi, M.S., Lee, J.S., Weigel, D. and Ahn, J.H. (2005) The 35S promoter used in a selectable marker gene of a plant transformation vector affects the expression of the transgene. *Planta* 221(4):523-530.

ATTACHMENT 3

SUMMARY OF PUBLIC SUBMISSIONS

1st Round

A total of 8 submissions were received – 7 from Australia and 1 from New Zealand.

1. Ricegrowers Limited (trading as SunRice)

- Strongly opposed to the approval of LLRICE62, primarily due to trade-related issues. The company claims that:

  - international market rejection of GM rice has prevented commercial production;
  - consumers have a preference for non-GM rice. Therefore, approval of LLRICE62 could adversely affect Australian rice exports, particularly where other competitor countries do not allow GM rice;
  - major compliance costs associated with testing, vendor assurance and other documentation increases the burden on Australian industry and could further erode the competitiveness of Australian rice exports;
  - the US is not a commercially viable production source of long-grain rice to Australia and New Zealand. Viable sources such as Thailand and Vietnam have strong anti-GM rice policies;
  - Australian and New Zealand businesses have rejected the use of GM foods;
  - approval in the Code is not the best way to manage accidental presence of GM commodities;
  - approval of GM rice could cause domestic consumers to perceive a higher risk associated with rice products which could in turn adversely affect purchasing behaviour of rice users in Australia and New Zealand.

- LLRICE62 offers no nutritional or other functional benefit to consumers or processors.
- Approval should be based on agreement by international safety experts, and this process represents a major burden for regulators in Australia/New Zealand.
- The EU system of assessment is more appropriate as it is based on the Precautionary Principle.
- Approval in other countries, for example the US, appears to be driven more by commercial interests in response to recent contamination events.
- Given that rice is an important staple food for many people, existing labelling laws would not adequately inform the consumer on the presence of GM rice.
- Stringent labelling of GM rice in food service channels would be necessary and must be properly enforced.

2. Australian Food and Grocery Council (AFGC)

- Supports approval of LLRICE62, contingent upon completion of a satisfactory safety assessment by FSANZ.
- In general, the AFGC supports a system of regulation for biotechnology products that applies appropriate standards of public health and safety and consideration for the environment.

- The recent review and international comparison of Australia's labelling requirements for GM foods found them to be appropriate and among the best in the world.
- Labelling of food products on the basis of the presence in the food of novel DNA or novel protein provides consumers with appropriate information on which to base an informed choice.

3. Victorian Department of Human Services

- No objection to this Application, seeking approval for LLRICE62, progressing to the next stage.

4. New South Wales Food Authority

- This Application should proceed.
- The costs of enforcement in monitoring for the presence of GM food should be considered in the benefit cost analysis. There could be a need for a National enforcement strategy for GM foods to reduce the burden of costs on individual States.

5. Ricegrowers' Association of Australia

- Strongly opposed to the Application. The approval of LLRICE62 should require much more stringent procedures and standards than are currently in place.
- Approval in the US is not dependent on stringent testing systems and can be obtained within 3 months.
- LLRICE62 is not grown commercially. In any case, it would not be able to compete in the Australian market with long grain rice from Vietnam and Thailand.
- Rice industries in all major rice exporting countries, including the US, have a policy to oppose commercial production of GM rice.
- LLRICE62 has no functional value (for example, health benefits through vitamin enrichment or iron fortification).
- Risk of accidental presence in the Australian food supply is not a good reason to apply for regulatory approval.
- If LLRICE62 is eventually approved, mandatory labelling should be imposed, so that consumers are made fully aware that the rice is GM. Labelling should be large and conspicuous and full disclosure by restaurants should also be required.
- If GM rice is approved in Australia, consumer confidence in rice products could be decimated because of the general consumer suspicion towards GM foods.
- As well as the domestic market, export markets could be severely damaged because of the loss of confidence in Australian producers as a source of non-GM rice.

6. Ivan Jeray

- Opposed to the approval of LLRICE62 because of safety concerns and a lack of proof that the crop is economically viable.
- Current labelling laws are inadequate and do not ensure consumers have sufficient information to avoid GM foods.

7. New Zealand Food Safety Authority

- Will provide comments after the Draft Assessment Report is released for consultation.

8. Food Technology Association of Victoria

- Supports Option 2 – to approve food derived from LLRICE62.

2nd Round

Seventeen submissions were received during the second consultation period.

1. Food Technology Association of Victoria

- Supports Option 2 – to approve food derived from LLRICE62.

2. New Zealand Food Safety Authority

- Supports Option 2 – to approve food derived from LLRICE62.

3. AFGC

- Supports Option 2 – to approve food derived from LLRICE62.
- The costs that might be imposed on the Australian rice industry if LLRICE62 is approved have been significantly underestimated in the Benefit/Cost analysis.
- FSANZ should consider an alternative regulatory option – to approve the importation of processed, manufactured food containing LLRICE62, instead of LLRICE62 as a raw commodity or rice products derived from LLRICE62.

4. New South Wales Food Authority

- Supports Option 2 – to approve food derived from LLRICE62, pending further consideration of the cost to government in enforcing GM food standards.
- Because of the complexity and expense involved in the analysis of GM foods, special consideration should apply to the cost burden on government enforcement agencies when assessing GM food applications.
- The documentation or paper trail to assist industry with GM food labelling is not obligatory and therefore enforcement must rely on food analysis.

5. Queensland Government

- Seeks detailed advice on how FSANZ determined the enforcement costs relating to this Application.

6. Paul Elwell-Sutton

- Strongly opposed to the approval of this Application.
- The labelling requirements for GM foods are not sufficiently robust to allow consumers to exercise their right to be fully informed because labelling of GM foods only depends on the presence of heritable material or protein.

7. Ivan Jeray

- Strongly opposed to the approval of this Application because of concerns about food and environmental safety, and on economic and ethical grounds.
- FSANZ cannot guarantee food safety or the safety of the herbicide glufosinate ammonium.
- FSANZ cannot guarantee that GM rice will not contaminate the environment and potentially destroy non-GM markets.
- FSANZ cannot guarantee and enforce the labelling of this product, so that consumers who are opposed to GM foods can avoid them.

8. GE Free Kaipara

- Strongly opposed to all genetically modified foods.
- Claims that the technology is experimental and will have far reaching and irreversible consequences.
- FSANZ should act responsibly and not bend to pressure from the biotechnology companies.
- There are better ways of [plant] breeding; organics is the way to go.

9. Ricegrowers' Association of Australia Inc.

- Strongly opposed to the approval of food derived from LLRICE62 in Australia.
- Australian rice competes in world markets because of its quality and 'clean, green, non-GM' status.
- Growers have invested heavily in the rice industry which is of great importance to the economy of the Riverina region.
- The FSANZ charter is focussed on food safety and there appears to be no mechanism for agricultural concerns to be examined meaningfully.
- The Draft Assessment Report has failed to address any of the concerns raised by the RiceGrowers Association and even fails to acknowledge that the agricultural sector is an affected party. Instead, FSANZ has put forward an unsubstantiated, philosophical view that GM rice would be good for the industry.
- Confidence in rice exports plummeted when consignments of rice from the USA were found to contain GM rice[7]. Rigorous and costly testing is now required by countries such as Japan and South Korea. Other countries imposed severe conditions on US rice which has added a costly compliance burden to the industry. This substantiates the claims that GM rice has an adverse impact on the marketability of rice to other countries.

[7] A GM rice known as LLRICE601 (Bayer CropScience) was found in 2006 at low levels in commercial long grain rice grown in the USA. The rice had only been grown experimentally and was not approved in any country including the US and therefore consignments of long grain rice required testing for the presence of the unapproved LLRICE62.

- The FSANZ Act demands that in varying standards, FSANZ must also have regard to the desirability of an efficient and internationally competitive food industry. Approval of LLRICE62 will damage the efficiency and international competitiveness of the Australian rice industry.
- The assessment should consider the potential impact on the Australian rice industry rather than on US growers.
- Australia is not a natural market for US rice and a rejection of this Application will have no impact on the commercialisation potential of this variety in the US. Therefore, trade in rice will not be hampered.

10. Ricegrowers Limited (trading as SunRice)

- Strongly opposed to the approval of food derived from LLRICE62 in Australia and submitted a detailed critique of the DAR.
- Claims that the DAR contains major omissions and errors of fact in relation to the potential benefits and costs of approving the Application to Australian consumers, regulatory bodies and the Australian rice industry.
- The benefit/cost analysis is not quantitative and is devoid of rigour because it offers only a superficial comparison of options.
- The regulatory impact assessment in the DAR is inadequate and does not comply with guidelines provided by the Office of Best Practice Regulation. FSANZ has therefore failed to establish a net benefit to Australia in approving this Application.
- Australian agriculture and the export industry have not been recognised as 'affected parties', while potential benefits to US rice growers have been considered.
- FSANZ has apparently ignored the substantial information provided by Ricegrowers Ltd in relation to:

    - the fact that LLRICE62 is not commercialised;
    - the absence of a commercially viable market in Australia;
    - the costs faced by Australian food safety regulatory bodies and AQIS;
    - the imposition on the Australian rice industry of compliance costs in some key export markets, as evidenced by the costs faced for export of medium grain rice from California;
    - the damage to Australia's image as a clean, green, non-GM exporter of rice; and
    - the potential to adversely impact on perceptions of rice products by Australian consumers, which could lower prices.

- Expresses residual concerns about the confidence FSANZ places in the Applicant's data (absence of independent, third-party verification).
- Considers that FSANZ vehemently defended the current safety assessment process for staple cereals such as rice, while at the same time is exploring the need for animal feeding studies.
- Contrary to FSANZ claims, Option 1 (no approval) offers benefits to consumers wishing to avoid GM foods because labelling requirements are inadequate and erode consumers right to 'informed choice'.
- Before recommending approval of this Application, FSANZ should comprehensively readdress the Regulatory Impact Assessment, taking the significant criticisms of the DAR presented by Ricegrowers and the Association into consideration.

- Claims that 'reluctant' importers of Australian rice products (e.g. Japan) would be likely to increase the burden of testing if GM rice is approved. This has already occurred in the US, where it was insisted that testing of medium grain rice was necessary when only long grain rice was affected by the adventitious presence of unapproved GM rice. This occurred despite there being no actual risk to medium grain rice due to the geographical separation of medium and long grain production systems.

11. Riviana Foods Pty Ltd

- Strongly opposed to the approval of food derived from LLRICE62 in Australia.
- Claims that rice products have broad penetration in the human diet and therefore approval of LLRICE62 should only be considered once much more stringent assessment procedures and test methods are in place.
- Claims that LLRICE62 offers no additional choice to consumers and has no overall public health benefits such as fortification or enrichment.
- Claims that consumers in the domestic market do not have a positive perception of GM foods and approval of LLRICE62 could lead to a loss of confidence in rice products. Labelling requirements will add to the negative impact of GM rice.
- Export markets are intolerant of GM foods.
- Gluten intolerant consumers rely heavily on rice based products. GM rice could lead to a loss of confidence in the gluten-free product market because these consumers are particularly health conscious, are likely to have a negative opinion on GM foods and will not be able to discriminate between GM and non-GM rice.
- Approval of LLRICE62 could thus lead to dire economic consequences for Australia's valuable rice food industry both domestically and internationally.

12. Vyt Vilkaitis

- Strongly opposed to the approval of food derived from LLRICE62 in Australia. Food and environmental safety should be the emphasis of the assessment.

13. Physicians and Scientists for Responsible Genetics (New Zealand)

- Opposed to the approval of LLRICE62 in New Zealand.
- Insufficient research has been carried out, particularly in relation to foreign proteins and the possibility of food allergies. A huge increase in soy allergies may be due to the GE protein present in Roundup Ready soy.
- A protein may be more allergenic due to misfolding, attached molecular chains, or rearrangement of unstable transgenes, but there is currently insufficient data to support or rule out these possibilities.
- It is possible that changes in GE soy DNA may produce new allergens. Unpredicted changes in the DNA were discovered years after it was on the market. The RNA produced is completely unexpected, and this could lead to the production of some unknown allergen.
- Other studies in both humans and mice show that GE soy could be at higher risk of causing allergies.
- Thousands of consumers in the U.S. complained to food manufacturers about possible reactions to StarLink corn.

- Recent research on plant-based transgenic vaccines found that minute quantities of a bacterial protein in GE corn provoke immune reactions in mice.
- Rice products are widely used in processed foods, particularly those consumed on a daily basis by people with gluten intolerance.
- The molecular characterisation of LLRICE62 is incomplete because it does not definitively rule out the possibility that it contains additional novel genes or novel fusion proteins.
- Glufosinate ammonium residues are likely because the plants are tolerant to this herbicide. This could lead to defects in human embryos.
- Increased herbicide residues present in GE foods e.g. Roundup Ready soy, might contribute to increased allergies.
- Bayer CropScience Pty Ltd has an abysmal record with respect to careful and thorough scientific practices, as evidenced by the contamination of rice in the United States with LLRice601.
- There has been no long-term, peer-reviewed studies proving that the consumption of transgenic food is safe.
- Supports a mandatory traceability system (Identity Preservation) for all foodstuffs to ensure that labelling accurately reflects the presence or absence of food or ingredients produced using Genetic engineering technology, to allow food withdrawals should unforeseen adverse effects to human health or the environment occur.
- Supports full disclosure to the public of all government information on residues in food of pesticides, herbicides or insecticides, heavy metals, industrial chemicals or their by-products, veterinary medicines and any other contaminants.

14. Greenpeace (Jeremy Tagar)

- Opposed to the approval of LLRICE62.
- Claims that FSANZ assessment standards are far below best practice in science.
- FSANZ has never rejected a GM food, nor reversed a decision to approve, and engages in deeply political and scientifically unjustifiable practices.
- Claims that FSANZ fails to adhere to its own primary objectives and instead pays greater attention to economic considerations that favour companies such as Bayer.
- FSANZ was not required to provide advice to CSIRO concerning safety data in GM peas, but did so anyway[8]. However, FSANZ has clearly not required that a similar set of studies be done on GM rice.
- FSANZ appears to be the only regulator satisfied with the data provided by Bayer. EFSA has requested additional information from Bayer that does not appear to have been supplied several years later.
- The assessment should not rely on industry generated data as this is notoriously unreliable.
- In the safety assessment, FSANZ failed to consider the PAT protein as it is produced in LLRICE62.

[8] CSIRO requested advice from FSANZ in relation to studies that would be required to undertake a safety assessment of GM peas that were in the research and development phase. CSIRO commissioned certain studies in mice to be conducted at the John Curtin School of Medical Research, Canberra. The results of these studies indicated an immune response to the injected peas in mice.

- Use of compositional analyses to determine similarity as a proxy for actual safety studies is scientifically unjustifiable. There has been no screening for unintended changes using proteomics or mRNA analysis, and no explanation provided as to why these data have not been used in the assessment.
- The anticipated intake/extent of use factor is relevant. Asian people in Australia may have much higher levels of consumption.
- The cost benefit analysis is deeply biased and flawed.
- FSANZ has not explored the potential adverse environmental effects.

15. Adrian Piccoli MP (Nationals; NSW Shadow Minister for Natural Resources)

- Supports the Australian rice industry's opposition to LLRICE62.
- LLRICE 62 offers no benefits to consumers and exposes the rice industry in Australia to potential trade disruptions and compliance costs.
- The rice industry is economically important to New South Wales and continues to compete in the world rice market, however approval of LLRICE62 could represent a huge risk to the viability of Australian rice producers.

16. Tony Catanzariti MLC (NSW; Country Labor)

- Would not support anything that has the capacity to damage the integrity of the Australian rice industry.
- The Australian rice industry is a major contributor to the regional economy of Southern NSW and prides itself on its 'clean, green' image.
- It is important that consumers are properly informed about the food they purchase as many prefer non-GM foods.

17. Kay Hull MP (Nationals; Federal Member for Riverina)

- Supports the Australian rice industry and its opposition to GM rice LLRICE62.
- The Australian rice industry performs in a competitive world market and must operate at the lowest possible costs to survive.
- Approval of LLRICE62 will not deliver any commercial or functional benefit to consumers but could threaten Australia's ability to trade in non-GM rice.
- When US rice exports were found to contain unapproved GM rice [LLrice601], trade was severely affected and a costly compliance burden was imposed on US producers.
- The drought has already had a devastating effect on the Australian rice industry.
- The cost-benefit analysis needs to give due consideration to the possible impacts on Australian consumers and the rice industry.
- Any approval for LLRICE62 is premature as US rice growers do not want to grow GM rice.

18. John Williams MP (Nationals; Federal Member for Murray Darling)

- Supports the Australian rice industry's opposition to the importation of GM rice into Australia.
- The major concern of the rice industry is the cost benefit analysis in the Draft Assessment Report, because it did not consider the potential disruption to trade and burden of additional compliance costs on the industry.

- GM rice offers no benefits to consumers.
- It is extremely unlikely that LLRICE62 would be grown in the US and exported to Australia over the next five years, so any approval seems premature.

19. National Farmers' Federation

- Expresses support for the Australian rice industry, however is not opposed to GM foods or GM crops, and supports farmers' right to choose a production method best suited to their business needs.
- The claim made by FSANZ in the Draft Assessment Report, that additional compliance costs would be minimal if LLRICE62 was approved, cannot be substantiated.
- US rice suppliers were burdened by significantly increased compliance costs following detection in 2006 of LLrice601 in US rice exports.
- GM rice is not yet commercialised and is unlikely to be accepted by markets.
- LLRICE62 offers no benefits for consumers.