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

6-07
3 October 2007

DRAFT ASSESSMENT REPORT

APPLICATION A589

FOOD DERIVED FROM GLUFOSINATE AMMONIUM-TOLERANT RICE LLRICE62

DEADLINE FOR PUBLIC SUBMISSIONS: 6pm (Canberra time) 14 November 2007
SUBMISSIONS RECEIVED AFTER THIS DEADLINE
WILL NOT BE CONSIDERED
(See 'Invitation for Public Submissions' for details)

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 in the Code. 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 grain. Some processed derivatives such as rice bran oil contain no plant proteins.

Labelling addresses the requirement of section 18(1)(b) of the Act; 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 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 to all sectors outweigh the costs associated with the approval.

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.

Preferred Approach

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 Preferred Approach

An amendment to the Code approving food derived from glufosinate ammonium tolerant rice LLRICE62 in Australia and New Zealand is recommended 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 Assessment was advertised for public comment between 13 December 2006 and 7 February 2007. A total of eight submissions were received during this period and a summary of these is attached to this Report.

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

Public submissions will be invited on this Draft Assessment Report.

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INVITATION FOR PUBLIC SUBMISSIONS

FSANZ invites public comment on this Draft Assessment Report for the purpose of preparing an amendment to the Code for approval by the FSANZ Board.

Written submissions are invited from interested individuals and organisations to assist FSANZ in preparing the Final Assessment of this Application. Submissions should, where possible, address the objectives of FSANZ as set out in section 18 of the FSANZ Act. Information providing details of potential costs and benefits of the proposed change to the Code from stakeholders is highly desirable. Claims made in submissions should be supported wherever possible by referencing or including relevant studies, research findings, trials, surveys etc. Technical information should be in sufficient detail to allow independent scientific assessment.

The processes of FSANZ are open to public scrutiny, and any submissions received will ordinarily be placed on the public register of FSANZ and made available for inspection. If you wish any information contained in a submission to remain confidential to FSANZ, you should clearly identify the sensitive information and provide justification for treating it as confidential commercial information. Section 114 of the FSANZ Act requires FSANZ to treat in-confidence, trade secrets relating to food and any other information relating to food, the commercial value of which would be, or could reasonably be expected to be, destroyed or diminished by disclosure.

Submissions must be made in writing and should clearly be marked with the word 'Submission' and quote the correct project number and name. Submissions may be sent to one of the following addresses:

Food Standards Australia New Zealand
PO Box 7186
Canberra BC ACT 2610
AUSTRALIA
Tel (02) 6271 2222
www.foodstandards.gov.au

Food Standards Australia New Zealand
PO Box 10559
The Terrace WELLINGTON 6036
NEW ZEALAND
Tel (04) 473 9942
www.foodstandards.govt.nz

Submissions need to be received by FSANZ by 6pm (Canberra time) 14 November 2007.

Submissions received after this date will not be considered, unless agreement for an extension has been given prior to this closing date. Agreement to an extension of time will only be given if extraordinary circumstances warrant an extension to the submission period. Any agreed extension will be notified on the FSANZ website and will apply to all submitters.

While FSANZ accepts submissions in hard copy to our offices, it is more convenient and quicker to receive submissions electronically through the FSANZ website using the Standards Development tab and then through Documents for Public Comment. Questions relating to making submissions or the application process can be directed to the Standards Management Officer at the above address or by emailing standards.management@foodstandards.gov.au.

Assessment reports are available for viewing and downloading from the FSANZ website. Alternatively, requests for paper copies of reports or other general inquiries can be directed to FSANZ's Information Officer at either of the above addresses or by emailing info@foodstandards.gov.au.

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 LLRICE 62, under Standard 1.5.2 – Food produced using Gene Technology.

This Draft Assessment includes a full scientific evaluation of LLRICE 62 according to FSANZ guidelines[1], to assess its safety for human consumption. Public comment is now sought on the safety assessment and proposed recommendations prior to a Final Assessment and completion of the Application.

1. Background

LLRICE 62 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.

LLRICE 62 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 USA (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 in the Code 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 LLRICE 62, a variety of GM rice tolerant to the herbicide glufosinate ammonium. Although commercial release of the grain will be in overseas countries, there is a possibility that imported rice products could include LLRICE62. The Applicant is therefore seeking an amendment to Standard 1.5.2 to approve food derived from LLRICE 62 in Australian and New Zealand markets.

Food derived from LLRICE 62 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).

[1] FSANZ (2005) Guidelines for the Safety Assessment of Genetically Modified Foods

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 LLRICE 62 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 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.

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 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.

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 for this Application. The two regulatory options available for this Application are:

6.1 Option 1 – Prohibit food from LLRICE 62

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

6.2 Option 2 – Approve food from LLRICE 62

Amend Standard 1.5.2 of the Code 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 may include the following:

- Consumers, particularly those who have concerns about biotechnology;
- Food importers and distributors of wholesale ingredients;
- The manufacturing and retail sectors of the food industry; 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 LLRICE 62 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 all sectors of 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 costs and benefits of the regulation, and its health, economic and social impacts.

7.2.1 Option 1 – prohibit food derived from LLRICE62

Consumers: Possible restriction in the availability of rice products if LLRICE62 is present in imported foods.
No impact on consumers wishing to avoid GM foods, as food from glufosinate ammonium-tolerant rice is not currently permitted in the food supply.

Government: Potential impact if considered inconsistent with WTO obligations but impact would be in terms of trade policy rather than in government revenue.

Industry: Possible restriction on rice imports if LLRICE62 is commercialised overseas. Potential longer-term impact - any successful WTO challenge has the potential to impact adversely on food industry.

7.2.2 Option 2 – approve food derived from LLRICE62

Consumers: No restriction on imported rice products if derived from LLRICE62. Increased choice in the marketplace as a result of mandatory labelling of most products derived from LLRICE62.
Potential impact on consumers wishing to avoid GM rice by a possible restriction of choice of products, or increased prices for non-GM rice.

Government: Benefit that if LLRICE62 was detected in rice imports, approval would ensure compliance of those products with the Code. This would ensure no potential for trade disruption on regulatory grounds.
Approval of LLRICE62 would ensure no 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.

Industry: Broader market access and increased choice in raw materials for food manufacturing. Benefit to importers of processed foods containing rice as an ingredient as foods derived from LLRICE62 would be compliant with the Code.
Possible cost to food industry as some food ingredients derived from LLRICE62 would 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 varieties of rice, option 1 is likely to be inconsistent with Australia and New Zealand's WTO obligations. Option 1 would also offer little benefit to consumers wishing to avoid GM foods, as approval of LLRICE62 in other countries could limit supplementation of the Australian and New Zealand market with imported rice products. Once GM rice is commercialised and enters international markets, industry costs associated with quality assurance documentation would be independent of food approval in Australia and New Zealand. Primary producers would benefit from an increased choice of crop lines with potentially lower production costs and higher yields, which could flow on to other sectors including consumers in Australia and New Zealand as lower food prices.

As LLRICE62 has been found to be safe for human consumption and the potential benefits outweigh the potential costs, Option 2, an amendment to Standard 1.5.2 of the Code giving approval to food from glufosinate ammonium-tolerant rice LLRICE62, is therefore the preferred option.

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 of the Code. 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 of the Code 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 Standards, 2007* can be legally sold in Australia.

COMMUNICATION

9. Communication and Consultation Strategy

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. In addition, FSANZ will issue a media release drawing journalists' attention to the matter.

As normally applies to all GM food assessments, the Draft Assessment Report for this Application will be available to the public on the FSANZ website and distributed to major stakeholders. Public comment on this Draft Assessment will be sought prior to preparation of the Final Assessment Report.

The Applicant and individuals and organisations who make submissions on this Application will be notified at each stage of the Application. After the FSANZ Board has considered the Final Assessment Report, if the Application is approved, we will notify the Ministerial Council. If the approval of food derived from glufosinate ammonium tolerant 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.

10. Consultation

10.1 Public consultation

The Initial Assessment was advertised for public comment between 13 December 2006 and 7 February 2007. Eight submissions were received during this period and a summary of these is included in **Attachment 3** to this Report.

Australian rice growers have expressed opposition to the approval of LLRICE62 rice on commercial grounds. FSANZ has discussed market-related issues with other areas of government and with the rice industry in Australia as major stakeholders.

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

10.1.1 Potential impact on Australia's export trade

Ricegrowers Limited (trading as SunRice) and an industry body, the Ricegrowers' Association of Australia, are strongly opposed to the application on the grounds that approval of GM rice could significantly affect Australian rice exports, particularly to countries that reject GM products.

If approval for LLRICE62 went ahead, it is further claimed that significant costs could be involved in testing and generating quality assurance statements and other documentation for the export market, resulting in a major economic impact on the industry as a whole.

10.1.1.1 Response

The Applicant has applied for approval of LLRICE62 in several South-East Asian countries in addition to Australia and New Zealand. Approval as food has already been obtained in Argentina (2006), the Russian Federation (2003), the US (2000) and Canada (2006). Regulatory approval in a number of countries is necessary before commercialisation of LLRICE62 can occur because of global trade in rice products. Whole rice grain imported from the US is typically non-viable parboiled rice with no potential for cultivation and therefore the Applicant has not sought environmental approval in Australia from the Office of the Gene Technology Regulator (OGTR).

Food regulatory approval for LLRICE62 would be necessary to ensure that any processed rice products imported to Australia from any of the countries where LLRICE62 is currently approved would be lawful. Products with rice derivatives could involve a large number of processed foods including breakfast cereals and bakery products, to name a few. To ensure continuing availability and trade in such products after LLRICE62 is commercialised, regulatory approval in Australia and New Zealand is necessary.

Current guidelines on the labelling requirements for GM foods entering the country as imports. This would also apply to rice products derived from LLRICE62. Importers and manufacturers involved in the food industry would continue to be able to source non-GM whole rice or derivatives if preferred.

FSANZ acknowledges that Australian rice exports are currently not associated with GM products. However, approval of GM rice in Australia and New Zealand would be only one element of negotiations between companies involved in international trade and would be expected to have negligible impact on business compliance costs. Further, approval of LLRICE62 in Australia and New Zealand would not necessarily compromise trade in non-GM rice, particularly as GM rice is currently not grown in either country. In the event of international market penetration of GM rice, a price premium may apply to non-GM varieties.

10.1.2 Potential impact on sales in the domestic market

Opponents of this Application contend that LLRICE62 offers no consumer benefit and could adversely affect the domestic market, particularly for consumers who remain wary of GM foods.

10.1.2.1 Response

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 will be non-GM rice. As a result, consumers in Australia and New Zealand would have reasonable choice in the marketplace.

To date, approved GM commodities contain traits of agronomic importance; those that offer potential benefits to primary producers of the commodity. Lowering production costs for farmers through the delivery of GM crops is claimed to have a flow-on beneficial effect on consumers through pricing of retail products. A number of GM foods that offer nutritional benefits to consumers are currently under development, however none of these are commercialised lines at this time.

10.1.3 Enforcement costs

The NSW Food Authority raised the issue of rising enforcement costs for government in terms of available resources, labour and reagent costs. The Authority considers that a national enforcement strategy for GM food approvals could be needed to address these issues.

10.1.3.1 Response

The costs associated with detecting GM from non-GM sources depend on the level of detail required for the investigation, as the number of introduced genetic traits is relatively small compared to the number of individually approved GM lines. Routine detection methods can distinguish a GM from a non-GM source when genetic material is present, however other analyses could be required for event-specific detection.

Guidelines to assist industry with compliance costs associated with labelling requirements under Standard 1.5.2 call for food manufacturers to seek and maintain documentation relating to the GM status of individual ingredients used in their products. In approving the expanded labelling requirements for GM foods in 2000, Health Ministers indicated that the purpose of the paper trail was to reduce the reliance on laboratory testing of foods as the sole enforcement tool.

Costs associated with the enforcement by jurisdictions of any new food regulatory measure are considered by FSANZ in the benefit cost analysis and are not unique to GM foods. Inevitably, enforcement costs would be expected to rise over time as a result of the need to regulate an ever-increasing number of new food additives, processing aids and novel technologies in the Code. Australia and New Zealand's current system of food regulation provides for the discussion of such issues by the Implementation Sub-Committee (ISC).

10.2 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 LLRICE 62 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 will be 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 will enable other WTO member countries to comment on proposed changes to standards where they may have a significant impact on them.

CONCLUSION

11. Conclusion and Preferred Option

An amendment to the Code to give approval to the sale and use of food derived from rice line LLRICE62 in Australia and New Zealand is proposed on the basis that food derived from glufosinate ammonium tolerant rice LLRICE62 is as safe for human consumption as food from conventional rice varieties.

12. 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. Draft Safety Assessment Report for glufosinate ammonium tolerant rice LLRICE62
3. Summary of first round public submissions

ATTACHMENT 1

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

Section 94 of the FSANZ Act provides that standards or variations to standards are legislative instruments, but 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

APPLICATION A589: FOOD DERIVED FROM GLUFOSINATE AMMONIUM-TOLERANT RICE LLRICE62

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 1900s. 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 1502bp 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
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 2665bp 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 149bp of rice genomic DNA, while the 5' flanking sequence consisted of 669bp 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 (173301bp) was identified. Apart from a deletion of 18bp 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 kilobases (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[2], and as an enzyme which confers natural resistance (Kumada, 1988).

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.

[2] 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 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.

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.

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[3].

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[4]. 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).

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

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.

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[5]. 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.

[5] 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.

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. 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).

5.1 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.

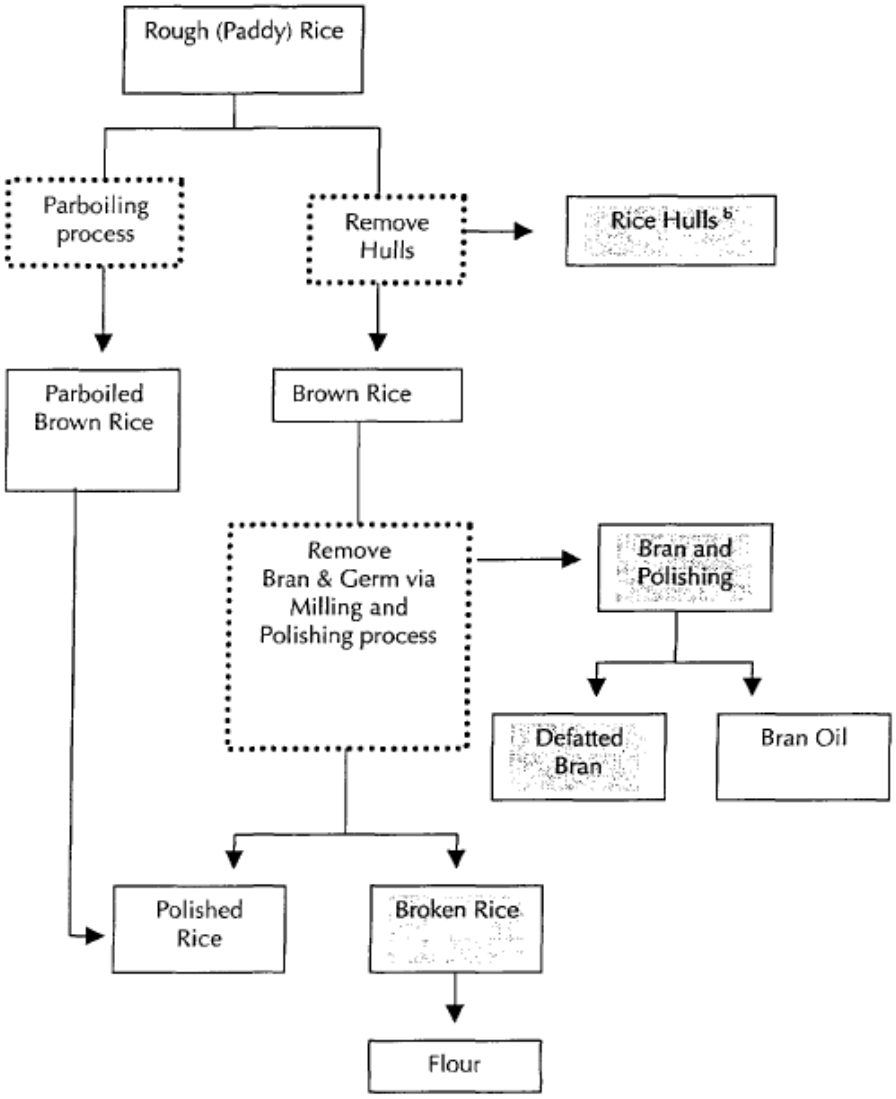

Figure 1: Rice Processing (outlined in broken lines) and Products (outlined in solid lines) (Amann, 1998)

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.

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ATTACHMENT 3

SUMMARY OF FIRST ROUND PUBLIC SUBMISSIONS

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, seeking approval for LLRICE62, 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.