Notification C/GB/03/M5/3

Glufosinate-tolerant Rice
Transformation event LLRICE62
Summary Information Format

Completed on
14 August 2003

Submitted on
22 August 2003

SUMMARY INFORMATION FORMAT FOR PRODUCTS CONTAINING GENETICALLY MODIFIED HIGHER PLANTS (GMHPs)

A. GENERAL INFORMATION

1. Details of notification

a) Member State of notification: United Kingdom
b) Notification number C/GB/03/M5/3
c) Name of the product (commercial and other names): Grain of GM rice (*Oryza sativa*) lines with tolerance to glufosinate-ammonium derived by traditional breeding methods from crosses between GM rice transformation event LLRICE62 (OECD code ACS-OS002-5) and non-GM rice cultivars.
d) Date of acknowledgement of notification 26/8/03

2. Notifier

a) Name of notifier: Bayer CropScience Limited		
b) Address of notifier: Regulatory Affairs Manager Hauxton, Cambridge, CB2 5HU, UK tel. +44 (0)1223 252416		
c) Is the notifier	domestic manufacturer: No	importer: Yes
d) In the case of an import the name and address of the manufacturer shall be given Bayer CropScience L.P. 2 T.W. Alexander Drive Research Triangle Park NC 27709, USA		

3. General description of the product

a) Name of the recipient or parental plant and the intended function of the genetic modification The recipient is a commercial variety of rice, "Bengal". The intended function of the genetic modification is tolerance to glufosinate ammonium.
b) Any specific form in which the product must not be placed on the market (seeds, cut-flowers, vegetative parts, etc.) as a proposed condition of the authorisation applied for None
c) Intended use of the product and types of users Import in the EU for all uses as any other rice (food, feed and industrial uses).

d) Any specific instructions and/or recommendations for use, storage and handling, including mandatory restrictions proposed as a condition of the authorisation applied for

No mandatory restriction for use, storage and handling are proposed as a condition of the authorisation. All standard practices applicable to rice today remain adequate for the handling of glufosinate-tolerant varieties.

If genetically modified rice is co-mingled with non-genetically modified rice during use, storage and handling, the corresponding batch will have to be labelled according to the legislation in application to the EU.

e) If applicable, geographical areas within the EU to which the product is intended to be confined under the terms of the authorisation applied for

Not applicable.

f) Any type of environment to which the product is unsuited

None

g) Any proposed packaging requirements

Like any other rice.

h) Any proposed labelling requirements in addition to those required by law

Bayer CropScience certifies that in the growing counties, commercial sale containers and invoices for planting seed of LibertyLink rice varieties will clearly contain the language "This product contains Genetically Modified Organisms" and that a statement of the requirement for consumer labelling in some commercial markets will be provided to the purchaser of the planting seed.

The language of the label may be modified to comply with legal requirements of the Cartagena Protocol on Biosafety to the Convention on Biological Diversity and the Regulation (proposal 2001/0180/COD) of the European Community concerning traceability and labelling of GMO's and traceability of food and feed products produced from GMOs and amending Directive 2001/18/EC, as these initiatives come into force.

i) Estimated potential demand

a) in the Community:

For the last three years, ~25% of the EU's imported rice originated in the U.S. (800,000 metric tonnes of rice in the 2001/2002 market year). The United Kingdom, the Netherlands, Germany, France and Belgium are the principal importers of U.S. rice.

b) in export markets for EC supplies

Not relevant.

j) Unique identification code(s) of the GMO(s) Transformation event LLRICE62 : ACS-OS002-5

4. Has the GMHP referred to in this product been notified under Part B of Directive 2001/18/EC and/or Directive 90/220/EEC?

Yes ☐	No ☒
(i) If *no*, refer to risk analysis data on the basis of the elements of Part B of Directive 2001/18/EC Risk assessment information provided in following points 9-11, 14-27, 29-33.	

5. Is the product being simultaneously notified to another Member State ?

Yes ☐	No ☐
(i) If *no*, refer to risk analysis data on the basis of the elements of Part B of Directive 2001/18/EC	

Or

Has the product been notified in a third country either previously or simultaneously?

Yes ☒	No ☐
If *yes*, please specify - USA (commercial uses) - Canada (import) - Mexico (import) - Russian Federation (import) - Japan (import) - Brazil (commercial uses) - Argentina (commercial uses)	

6. Has the same GMHP been previously notified for marketing in the Community?

Yes ☐	No ☒
If *yes*, give notification number and Member State	

7. Measures suggested by the notifier to take in case of unintended release or misuse as well as measures for disposal and treatment

Milled rice grain is not viable seed. The qualities of the grain are the same as other rice in commerce and do not constitute an environmental hazard.

In the case of accidental spillage of LLRICE62 in transit or at the processing facility, the area will be monitored for one season for the germination and plant establishment of the spilled rice grain.

B. NATURE OF THE GMHP CONTAINED IN THE PRODUCT

INFORMATION RELATING TO THE RECIPIENT OR (WHERE APPROPRIATE) PARENTAL PLANTS

8. Complete name

a) Family name: *Poaceae*
b) Genus: *Oryza*
c) Species: *sativa*
d) Subspecies: *indica, japonica, javanica*
e) Cultivar/breeding line: Bengal
f) Common name: rice

9. a) Information concerning reproduction

(i) Mode(s) of reproduction

a) Autogamous, cultivated rice is basically propagated by seeds which are produced by self-pollination. Wind can move rice pollen, however the life span of pollen is short (3 to 5 min) and the most cultivated rice have stigma that do not extrude from the glums. A high rate of outcrossing has been observed in wild rices due to variation in the morphological characteristics of the flower.
b) Vegetative, may be propagated by tiller buds.

(ii) Specific factors affecting reproduction, if any

a) Self-pollination : Pollen formation and fertilization of rice are affected by low and high temperature, flooding and drought. Low temperature (15 to 20˚) affect the microspore stage and high temperature can affect anthesis.
b) Vegetative : favorable water and temperature conditions

(iii)Generation time: 4 to 6 months

9. b) Sexual compatibility with other cultivated or wild plant species

There is no evidence of genetic transfer and exchange with other organisms than those with which rice is able to produce fertile crosses through sexual reproduction.

In Europe, *O. sativa* is present in two forms; the cultivated rice in Italy, France, Portugal and Spain, and the introduced weed, red rice, which is present in some fields of cultivated rice.

10. Survivability

a) Ability to form structures for survival or dormancy

Rice is cultivated annually. Seed are formed as structures enhancing survival.
However rice plants can grow vegetatively and continuously under favourable water and temperature conditions. Rice is adapted to the humid tropics as a semi-aquatic plant.
Wild rice and older cultivars are noted for ease of seed shattering, however modern cultivars developed for machine harvesting have been selected for lack of shattering. Most cultivated rice has been selected for seed expressing a weak dormancy that can be broken with a short period of dry afterripening. Seed dormancy can be important to allow ripening of the grain and harvest without precocious germination. The panicles of red rice biotypes easily shatter and have strong seed dormancy, becoming a weed problem in rice fields. Red rice seed, when buried in the soil can remain viable for many years and thus create a recurring weed problem.

b) Specific factors affecting survivability, if any

As a vegetative plant, rice does not survive in dry, cold climates.

Seed survival is affected by soil conditions such as temperature and moisture content.

11. Dissemination

a) Ways and extent of dissemination

Three developmental stages are susceptible for dispersal: pollen, seed and vegetative.

- Pollen may be carried by the wind, however, the short life of pollen in cultivated rice (3 to 5 minutes) and the narrow window of female receptivity limit wind as a means of effective dissemination.
- Seed may be dispersed during transport, at sowing and essentially before and during harvest.
- Water seeded rice plants may be up-rooted by wind before the roots establish good contact with the soil. Up-rooted plants may be dispersed by wind on the water surface within the irrigation system.

b) Specific factors affecting dissemination, if any

Rice seed has no structural modifications to facilitate transfer by animals. Dissemination is mainly the result of human activity.

12. Geographical distribution of the plant

Rice is grown worldwide and is a staple food for half of the world's population. Annually about 530 million tons of rice are harvested from the fields of 146 million hectares world-wide. More than 90% of the world rice production comes from Asia, 5% from the Americas, 3% from Africa, and another 1% from Europe and Oceania.

The domestication of rice is considered to have likely occurred in the eastern part of India or in the Yunnan district of China and then widely distributed as a crop. Cultivated rice is recognized to have two varietal groups based upon adaptation, Indica and Japonica. In very general terms, the Indica form is more adapted to tropical, rain-fed agriculture. The Japonica form is found more in temperate, irrigated agriculture, however, Japonica rice is often grown in the upland fields of tropical regions. The two major grain types of rice produced in the USA (long and medium grain) are based upon Japonica germplasm. The preferred grain type of Brazil and Argentina is based upon Indica germplasm.

At present the northern limit of rice cultivation is the border of China and Russia (lat. 53°N.) and the southern limit, Australia (lat. 30°S) and central Argentina (lat. 51°S.) Rice can be adapted to environmental extremes, however US rice varieties are dependent upon irrigation and temperate climates. The northern limit of US rice cultivation is lat. 39°N.

13. In the case of plant species not normally grown in the Member State(s), description of the natural habitat of the plant, including information on natural predators, parasites, competitors and symbionts

Rice is cultivated by the Member States of Italy, Spain, Greece, Portugal and France (listed in order of volume). Italy and Spain represent the primary regions for irrigated rice production in Europe.

Rice is found in a variety of ecosystems. Natural habitats of wild rice are swampy places, such as ditches, irrigation canals, or marshlands. The perennial types are found in deep water, while annual types grow in seasonal swamps. The distribution of so called low land and upland rices can overlap. They have been found in the same marsh, but have different niches in the same habitat. In Asia and West Africa, where rice has been cultivated since ancient times, wild rice populations inhabit disturbed areas. In tropical America and Australia, rice remains in a natural state. The most common predator of rice habitat is man. Draining of wetlands to build housing has destroyed wild rice habitat in Asia, Africa and the Americas. Sensitivity to photoperiods and tolerance to low temperature, drought, flooding, salt and parasites can dictate the habitats of rice. Ecotypes of wild rice include lowland, upland, deep-water and floating. Cultivated rice habitats are either irrigated or upland, rainfed.

14. Potentially significant interactions of the plant with other organisms in the ecosystem where it is usually grown, including information on toxic effects on humans, animals and other organisms

There are no major interactions with the environment except for being a crop.
Rice does not produce nectar however, it can be visited by foraging insects to collect pollen. A number of insects (e.g. rice water weevil, *Leptocorixa. oryzophilus*; rice stink bug, *Oebalus pugnax* ; grasshoppers, *Conocephalus fasciatus* and *Melanopsis differentialis*; rice stalk borer, *Chilo plejadellus*) and diseases (e.g. sheath blight, *Rhizoctonia solani*; blast, *Pyricularia oryzae*; and stem rot, *Sclerotium oryzae*) may infest the crop.
The rice plant is not pathogenic or harmful, either living or dead.
Rice is used for food in various forms including whole and milled grain, flour and bran. Husks are used for fertilisers and animal feed, and straw for making various materials, e.g. mats.
Rice contains a small number of antinutritional factors, all of which are concentrated in the bran fraction. The antinutritional factors include phytic acid, trypsin inhibitor, and hemagglutinin (lectin). With the exception of phytic acid, all of the antinutritional factors are subject to heat denaturation. Rice bran is typically subjected to a dry or moist heat treatment to denature lipases that otherwise would cause the rice bran to become rancid very quickly.
Some segments of the population have been identified to have an allergenic reaction to rice. The major allergenic factor is a 16 kD globulin.

15. Phenotypic and genetic traits

The recipient organism is a commercial rice variety commonly cultivated in the United States (cultivar Bengal) with no specific phenotypic or genetic trait.

INFORMATION RELATING TO THE GENETIC MODIFICATION

16. Description of the methods used for the genetic modification

The transformation (DNA delivery) was performed by particle bombardment using a purified plasmid fragment containing the *bar* cassette.

17. Nature and source of the vector used

The plasmid, pB5/35S*bar*, is a derivative of the plasmid pUC19, which was derived from *Escherichia coli*.

18. Size, source [name of donor organism(s)] and intended function of each constituent fragment of the region intended for insertion

To obtain the transforming DNA, the plasmid pB5/35S*bar* was digested with restriction enzymes Pvul and HindIII (Figure 1), and the resulting restriction fragments were separated on an agarose gel. The cleavage position of the restriction enzymes in pB5/35S*bar* for HindIII is at nucleotide 2140 and for PvuI at nucleotide 3641. The 1502 bp HindIII/PvuI fragment contained the *bar* cassette (P35S-*bar*-T35S) and was purified from the gel, and used in transformation of the parental line, rice cultivar Bengal, to generate transformation event LLRICE62. The *nptIII* gene was not included in the transforming DNA fragment.
The size, source and intended function of each constituent are listed in Table 1.

Figure 1 Plasmid map of pB5/35S*bar*

The 1502bp HindIII/PvuI fragment of plasmid pB5/35S*bar*, used in the transformation, contains one open reading frame, *bar*, which is intact and functional in transformation event LLRICE62. Included on the map are the restriction endonucleases used in Southern blot analysis to demonstrate that a single copy and no plasmid backbone were inserted by the transformation process.

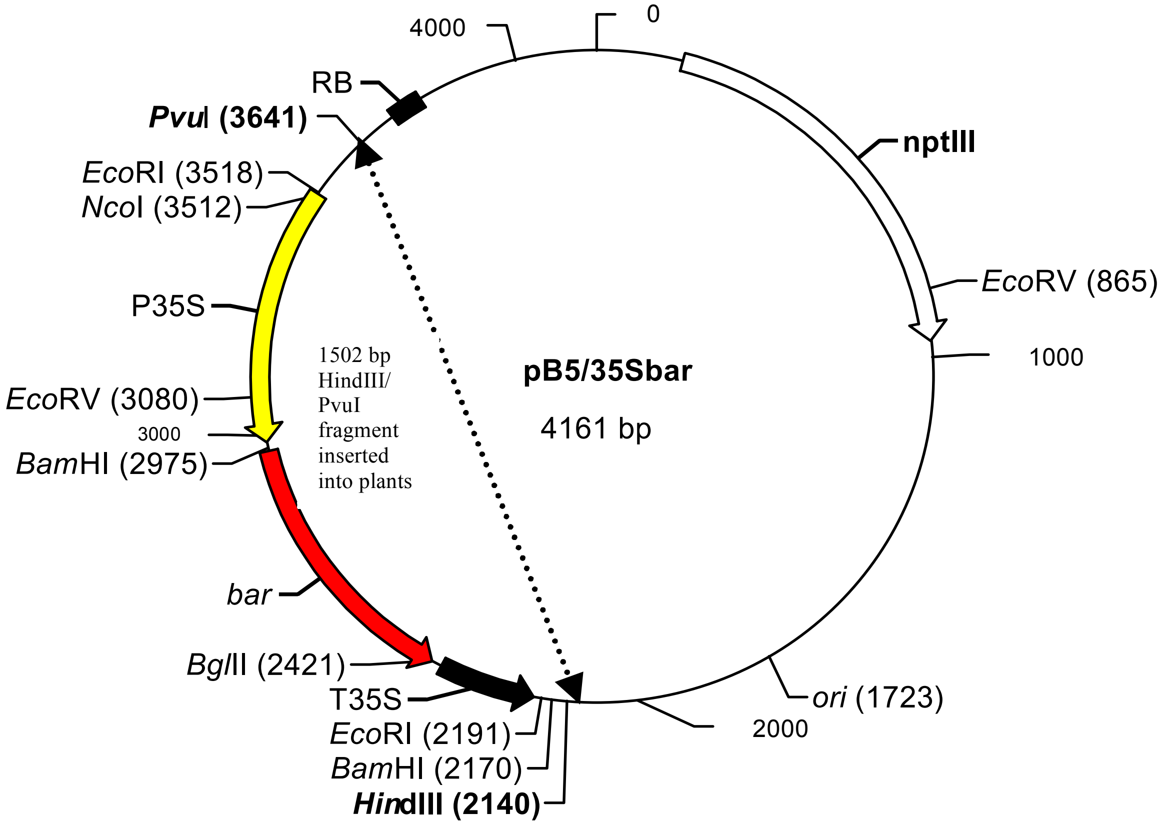

Table 1. Genetic elements of the inserted DNA component of the Vector pB5/35S*bar*

Abbre-viation	Definition	Source	Size (bp)	Accession nº or reference	Function
		Sequence derived from pUC19	56	Yanisch-Perron *et al.* 1985	Restriction site *Hind* III
	Polylinker sequence	Synthetic	9		Plasmid cloning site
T35S	Terminating signal of bar gene	Cauliflower Mosaic Virus	194	Franck, *et al.,* 1980; Pietrzak, *et al.,* 1986	Stop signal
	Polylinker sequence	Synthetic	19		Plasmid cloning site
bar	Glufosinate tolerance *bar* gene	*Sterptomyces hygroscopicus*	552	Thompson, *et al.,* 1987 X05822	Herbicide tolerance and selectable marker
	Polylinker sequence	Synthetic	16		Plasmid cloning site
P35S	Promoter	Cauliflower Mosaic Virus	532	Franck, *et al.,* 1980; Pietrzak, *et al.,* 1986	High level constitutive expression
		Sequence derived from pUC19	124	Yanisch-Perron *et al.* 1985	Restriction site *Pvu* I
			1502		

INFORMATION RELATING TO THE GMHP

19. Description of the trait(s) and characteristics which have been introduced or modified

Herbicide tolerance

Tolerance is based upon the *bar* gene, a bialophos resistance gene, isolated from the soil microorganism, *Streptomyces hygroscopicus*. Using recombinant DNA technologies, the *bar* gene has been cloned from *S. hygroscopicus*, fused with the 35S promoter from Cauliflower mosaic virus and introduced into the plant genome. The *bar* gene, when expressed, enables the production of the enzyme, Phosphinothricin-Acetyl-Transferase (PAT) that acetylates L-glufosinate and thereby conferring tolerance to herbicides based upon glufosinate ammonium.

20. Information on the sequences actually inserted/deleted/modified

a) Size and structure of the insert and methods used for its characterisation, including information on any parts of the vector introduced in the GMHP or any carrier or foreign DNA remaining in the GMHP

The inserted DNA sequence has a length of 1502 bp.

The determination of inserted sequences in event LLRICE62 confirmed the presence of one copy of the *bar* gene cassette. The DNA sequence of the insert is identical to the corresponding transforming plasmid DNA sequences. Southern blot hybridization data with genomic DNA cut with four different restriction enzymes demonstrate that the event LLRICE62 contains only one intact copy of the complete gene cassette.

Southern blot hybridization between genomic DNA of the event LLRICE62 and the vector DNA demonstrate the absence of any additional sequences from the vector used for the transformation.

b) In case of deletion, size and function of the deleted region(s)

A deletion of 18 base pairs was identified at the transgenic locus of LLRICE62

c) Location of the insert in the plant cells (integrated in the chromosome, chloroplast, mitochondrion, or maintained in a non-integrated form), and methods for its determination

Based upon Southern blot and genetic segregation analysis, it was demonstrated that the DNA has integrated in a single genetic locus in the rice nuclear genome (chromosomes).

Following analysis of the regions flanking the insert, there is no indication that insertion of the DNA has a negative or unintended effect.

d) Copy number and genetic stability of the insert

Southern blot, PCR and sequence analysis demonstrated that the glufosinate-tolerant, rice event LLRICE62 contains one copy of the *bar* gene cassette. To demonstrate the stability of the inserted DNA, Southern blot analysis was completed for different generations grown under different environmental conditions and for crosses into different genetic backgrounds.

The isolated DNA was digested with the EcoRV restriction enzyme, which has only one restriction recognition site in the transgene. Probing EcoRV restricted genomic DNA with the *bar* gene cassette showed the two expected bands in all rice event LLRICE62 samples. These bands represent the junctions between transgenic sequences and plant DNA sequences upstream and downstream of the insert and were identical in all samples.

The resulting Southern blots demonstrate the molecular stability of the rice event LLRICE62 at the genetic level over multiple generations, different locations and in four distinctive genetic backgrounds..

e) In case of modifications other than insertion or deletion, describe function of the modified genetic material before and after the modification as well as direct changes in expression of genes as a result of the modification

Not applicable

21. Information on the expression of the insert

a) Information on the expression of the insert and methods used for its characterisation

Linked to the plant promoter, 35S, the expression of the *bar* gene is targeted to green tissue of the plant. Expression level was measured by PAT protein specific ELISA. It was found that PAT protein constitutes 12 µg/g fresh weight of roots, 30 µg/g fresh weight of stems (culm) and 90 µg/g fresh weight of leaves. PAT protein comprises an average of 0.23, 0.19 and 0.13% of the total crude protein in roots, stems and leaves respectively, of rice event LLRICE62. ELISA reactive PAT protein was not found in the non-transgenic control rice organs. The limit of detection of the assay for the different matrices was 3.37 ng/g for roots, 0.22 ng/g for stems and 4.59 ng/g for leaves. Tissue samples were harvested from field-grown rice at the late vegetative/panicle development stage.

b) Parts of the plant where the insert is expressed (e.g. roots, stem, pollen, etc.)

From published experience with the 35S promoter in rice, we expected LLRICE62 plants to show high levels of PAT protein in the leaves and lesser amounts in the other organs. Indeed, we found the following order of PAT expression leaf blade>>stem (culm)>>roots, seed (grain) >>>>>>pollen (not detected). The amount of PAT protein in the leaves of LLRICE62 during the vegetative life cycle of the plant has an upper limit of approximately 150 µg/g fresh weight. The amount of PAT protein in seed is 12 µg/g fresh weight.

22. Information on how the GMHP differs from the recipient plant in

a) Mode(s) and/or rate of reproduction

Mode and rate of reproduction is by seed production and is the same as for conventional rice.

b) Dissemination

Three developmental stages are susceptible for dispersal: pollen, seed and vegetative. No differences in dissemination capacity have been observed between Genetically Modified LLRICE62 and non-genetically modified rice. Studies show that the genetic modification did not modify the characteristics of the rice that could impact dissemination.

- no difference in pollen characterises including; viability by vital stain, fertility in crosses as either a male or female parent, and floral structure.
- no difference in pollen dispersal to cultivate rice and to red rice under field conditions.
- no difference in seed morphology or fecundity measured as number of seed per panicle, grain biomass and 1000 seed weight.
- no difference in shattering, germination, length of dry afterripening requirement or dormancy as measured by standard laboratory rice seed physiology tests.
- no impact on tillering capacity that could be associated with an increase in vegetative dispersal as in certain rice wild species.

c) Survivability

For cultivated rice, survival is most determined by seed characteristics. There is no indication of changes in the seed characteristics as a result of the genetic modification. Rice seed left behind in a field after harvest can germinate the following season and become a “volunteer” in a subsequent crop. Studies show that the current agricultural practices are able to control both modified and unmodified volunteer rice plants.

d) Other differences

Rice varieties based upon transformation event LLRICE62 are tolerant to Liberty® herbicide, active ingredient glufosinate ammonium.

23. Potential for transfer of genetic material from the GMHP to other organisms

a) ***transfer of genetic material to other higher plants.***

Genetic transfer possible only to rice. There is no evidence of genetic transfer and exchange under natural condition with organisms other than those with which rice is able to produce fertile crosses through sexual reproduction. There are no indications that the potential for successful exchange of genetic material has changed due to the genetic modification.

- **Likelihood of gene flow.** Gene flow can occur into an adjacent rice crop and into weed red rice, however, the rate is likely to be very low because there exists a combination of genetic, botanical, geographic and agricultural barriers to gene flow. Gene flow will not occur into wild *Oryza* species, which are far removed from rice production.
- **Consequence of gene flow.** Furthermore, the transfer of the *bar* gene into red rice will not exacerbate problems of weed control or adversely impact agriculture. The fitness of crop-weed hybrids is not greater than that of red rice. No adverse impact to biodiversity was identified.

b) ***transfer of genetic material to bacteria.***

In order for any horizontal gene transfer to lead to a new type of micro-organism and therefore to introduce a significant impact, some of the following conditions will have to be fulfilled:

- the uptake should result in the incorporation of complete undegraded DNA
- the plant targeted genes should result in significant expression in a prokaryotic background
- the expression should represent a significant increase over the background level
- the traits should convey a competitive advantage to the strain in which they are incorporated.

In the very unlikely case where both horizontal gene transfer from genetically modified plants to bacteria would occur and where due to genetic recombination the genes would be expressed in micro-organisms (the bar gene is under the control of the 35S promoter, which is not functional in bacteria), this would have no impact since the transgene would not provide a selective advantage (the substrates for PAT protein is the herbicide glufosinate ammonium).

24. Information on any harmful effects on human health and the environment, arising from the genetic modification

No harmful effects on human health arising from the genetic modification are expected.

The PAT protein is not toxic for mammals and does not possess any of the characteristics associated with food allergens. Findings to support this conclusion include:

– The coding sequence of the *bar* gene is derived from a common soil microbe not known to be a pathogen.
– The PAT protein has no homology with any known allergens, toxins or antinutrients.
– The PAT protein has no glycosylation sites present on certain food allergens.
– The PAT protein is quickly degraded and denatured in gastric and intestinal fluids of domestic animals and humans.
– The PAT enzyme is highly substrate specific. It acts on its target, glufosinate but it does not act on glutamate, the closest structural analogue of L-glufosinate.
– There were no adverse effects found in mice, even at a high dose level of the PAT protein, after intravenous administration.

Rice grain of LLRICE62 has the same nutritional quality and identical allergen profile as rice in commerce. Safe consumption of the grain by humans is supported by the following comparisons:

- Nutritional compositions for whole grain and processed products of rice grain were not different from the standards of rice in commerce and for most parameters found to meet the standard of statistical equivalence.
- Antinutritional factors common to rice are well below acceptable levels.
- There is no increased risk of allergenic potential of the event LLRICE62 as compared to its counterpart in rice-allergic subjects.
- The amount of PAT protein present in LLRICE62 rice varieties is small. The amount of PAT protein detected in the grain and grain derived products contain less than 0.03% on a weight basis (w/w) of the crude protein. The PAT protein was not detectable in rice bran oil and parboiled brown rice.

25. Information on the safety of the GMHP to animal health, where the GMHP is intended to be used in animal feedstuffs, if different from that of the recipient/parental organism(s)

The primary use of rice is for human food, however rice grain and the by-products of rice milling are often included in animal diets. The nutritional composition of the grain and processed fractions (brown rice, parboiled brown rice, milled rice, bran, flour and rice bran oil) were found to be equivalent to other rice by chemical analysis.

To support the finding of nutritional equivalence, poultry and swine were fed diets containing rice under study conditions designed to evaluate growth and health parameters. Poultry were selected to evaluate the effects of a feed com ponent over an entire life span and under conditions of very rapid growth, thus the assay is highly sensitive for nutritional deficiencies or toxic effects. In contrast, swine represent a close approximation to the human food preferences and digestive processes. No differences were identified for nutritive value of the grain and no indications of toxic or adverse effects were associated with any of the sources of rice in either of the tested animal species.

The grain of transformation event LLRICE62 is not anti-nutritional or toxic for mammals and no effects on animal health are expected.

26. Mechanism of interaction between the GMHP and target organisms (if applicable) , if different from that of the recipient/parental organism(s)

Not applicable as there are no target organisms.

27. Potentially significant interactions with non-target organisms, if different from the recipient or parental organism(s)

There are no non-target organisms specific to the GMHP compared to non-genetically modified rice.

There are no observed effects of the herbicide-tolerant rice on non-target organisms:

- Field observations found no differences in insect populations or reactions to natural infestation of rice pathogens.
- No effect could be observed on birds or small mammals.

28. Description of detection and identification techniques for the GMHP, to distinguish it from the recipient or parental organism(s)

DNA-based methods:

A PCR protocol is available for rice event LLRICE62, based upon nucleotide sequences that are specific to the event. The protocol for this discriminating PCR (dPCR) has been provided to the British competent authority.

Protein-based methods:

The PAT protein present in LLRICE62 may be detected by an appropriate ELISA method.

INFORMATION ON THE POTENTIAL ENVIRONMENTAL IMPACT FROM THE RELEASE OF THE GMHP

29. Potential environmental impact from the release or the placing on the market of GMOs (Annex II, D2 of Directive 2001/18/EC), if different from a similar release or placing on the market of the recipient or parental organism(s)

The following conclusions were drawn (see items listed Annex II D2 of Directive 2001/18/EC):

1. Likelihood of the GMHP becoming more persistent than the recipient or parental plants in agricultural habitats or more invasive in natural habitats.

A review of the reproductive and vegetative fitness finds that LLRICE62 compares to its parent variety Bengal in all aspects except the tolerance to glufosinate ammonium herbicide. The PAT protein does not influence reproductive or vegetative fitness characters in rice or in other agricultural crops where it is in commercial use (corn, oilseed rape, cotton).

2. Any selective advantage or disadvantage conferred to the GMHP.

None. USDA concludes that PAT protein does not confer a selective advantage. Agronomic performance shows no disadvantage. The only circumstance in which a selective advantage could happen would be if some plants from escaped grain would be sprayed with glufosinate. As discussed below the likelihood that some escaped grain would germinate is very low because most of the imported grain is non-viable. In addition the herbicide glufosinate ammonium is not used in the vicinity of grain storage facilities, processing plants or roadways, areas where such an escape might occur.

3. Potential for gene transfer to the same or other sexually compatible plant species under conditions of planting the GMHP and any selective advantage or disadvantage conferred to those plant species.

Gene flow to red rice or other crop rice is possible in rice producing areas of Europe. Studies find the potential to be small. Impacts can be managed with modest isolation distances in commercial production. Prudent use of glufosinate ammonia herbicide can control red rice before it can flower, thus eliminating the opportunity of cross-pollination. In cases where red rice does flower in field production, subsequent monitoring has not found hybrids. The self-pollinating nature of rice and red rice serve as a barrier to cross pollination.

Nevertheless LLRICE62 is not intended to be grown in Europe and thus, this risk is only hypothetical.

4. Potential immediate and/or delayed environmental impact resulting from direct and indirect interactions between the GMHP and target organisms, such as predators, parasitoids, and pathogens (if applicable).

Potential impact is the same as for other commercial rice varieties. LLRICE62 is not a “pesticide plant” and there is no “target” organism. The target of the transgene is the herbicide, glufosinate ammonium. Therefore an assessment of this issue is not applicable.

5. Possible immediate and/or delayed environmental impact resulting from direct and indirect interactions of the GMHP with non-target organisms, (also taking into account organisms which interact with target organisms), including impact on population levels of competitors, herbivores, symbionts (where applicable), parasites and pathogens.

The extensive assessment of transformation event LLRICE62 in USA and Brazil has demonstrated that it behaves exactly as another rice.

6. Possible immediate and/or delayed effects on human health resulting from potential direct and indirect interactions of the GMHP and persons working with, coming into contact with or in the vicinity of the GMHP release(s).

Same as for other commercial rice varieties. Bayer CropScience safety assessment supports the benign toxicological profile of PAT protein. In addition, the PAT protein could be detected in rice pollen.

7. Possible immediate and/or delayed effects on animal health and consequences for the feed/food chain resulting from consumption of the GMO and any products derived from it, if it is intended to be used as animal feed.
No changes from commercial varieties. A thorough food and feed safety assessment has demonstrated that there is no change in the rice and by-products in terms of composition, and, hence, LLRICE62 is substantially equivalent to non GM rice except for the intended trait.

In addition, side by side comparison of diet prepared from LLRICE62 and conventional rice was made using two animal species, poultry and swine. The safety of LLRICE62 was confirmed by observation of the general health, well-being and typical growth of the animals. Performance indicators, such as growth and meat quality, confirmed the nutritional equivalence of LLRICE62 and conventional rice.
8. Possible immediate and/or delayed effects on biogeochemical processes resulting from potential direct and indirect interactions of the GMO and target and non-target organisms in the vicinity of the GMO release(s).
Same as for other commercial rice varieties.
9. Possible immediate and/or delayed, direct and indirect environmental impacts of the specific cultivation, management and harvesting techniques used for the GMHP where these are different from those used for non-GMHPs.
LLRICE62 will be imported for direct use as food and feed or for processing. No seeds will be imported for cultivation into the Europe. Therefore this question is not relevant. No change in the handling of the imported commodity.
The overall conclusion; there is no change in the handling of the imported commodity and no risk identified for the import of rice.

30. **Potential environmental impact of the interaction between the GMHP and target organisms (if applicable), if different from that of the recipient or parental organism(s)**

Not applicable, as there are no target organisms.

31. **Possible environmental impact resulting from potential interactions with non-target organisms, if different from that of the recipient or parental organism(s)**

a) Effects on biodiversity in the area of cultivation

Under pressure of selection in an area treated with glufosinate ammonium, LLRICE62 may establish in the environment and, thereby, modify the biodiversity. Furthermore it might transfer the trait via pollen flow to other cultivated rice or weed rice (red rice) or wild rice (*Oryza* sp.) in the vicinity and contribute to their establishment and modification of the biodiversity too. However the anticipated area of cultivation is not in Europe
Implications of the potential effects on biodiversity:
- Red rice in an introduced weed that resides only in agro-ecosystem and is not an important genetic resource for modern rice breeding.
- Floristic surveys have identified the distribution and habitats of the wild *Orzya* species. There is no overlap with wild species in temperate rice production, which include rice production in the US and Europe.
- No impact by *bar* gene or PAT protein on other organism that may consume rice grain.
- No change in nutritional composition or levels of anti-nutrients

b)Effects on biodiversity in other habitats LLRICE62 will be imported as mostly non-viable grain. Therefore the likelihood that some imported grain could escape from silos or lorries and germinate is very low. In addition, glufosinate ammonium is used in Europe only in agricultural areas (as a herbicide in grapevine and orchards, and as a desiccant in oilseed rape and potatoes). Therefore the very rare plants that might germinate would have no chance to be sprayed with glufosinate ammonium. Consequently the likelihood for LLRICE62 in the environment is not higher than conventional rice.
a) Effects on pollinators Rice is not pollinated by insects.
b) Effects on endangered species No adverse effects are expected on endangered species since no adverse effects have been observed on non-target organisms including birds and small mammals.

C. INFORMATION RELATING TO PREVIOUS RELEASES

32. History of previous releases notified under Part B of the Directive 2001/18/EC and under Part B of Directive 90/220/EEC by the same notifier

a) Notification number No releases of LLRICE62 have been made under Part B.
b) Conclusions of post-release monitoring No release in Europe, however in the USA, no persistent volunteers that could not be managed by current agricultural practice were observed.
c) Results of the release in respect to any risk to human health and the environment (submitted to the Competent Authority according to Article 10 of Directive 2001/18/EC) No release in Europe, however in the USA, no human health or environmental risks were observed.

33. History of previous releases carried out inside or outside the Community by the same notifier

Outside the Community **USA (field release since 1997, no longer regulated since 2000)** Authority overseeing the releases: Department of Agriculture Information on the releases at www.aphis.usda.gov/ **Brazil (field release since 1999)** Authority overseeing the releases: Comissão Técnica Nacional de Biossegurança (CTNBio) Information on the releases at http://www.mct.gov.br/ctnbio **Argentina (field release since 1998)** Authority overseeing the releases: CONABIA Information on the releases at http://siiap.sagyp.mecon.ar/http-hsi/english/conabia/frameing.htm

D INFORMATION RELATING TO THE MONITORING PLAN - IDENTIFIED TRAITS, CHARACTERISTICS AND UNCERTAINTIES RELATED TO THE GMO OR ITS INTERACTION WITH THE ENVIRONMENT THAT SHOULD BE ADDRESSED IN THE POST COMMERCIALISATION MONITORING PLAN

The environmental risk assessment identifies no risk for the import of rice.

LLRICE62 will be imported for direct use as food and feed or for processing. No seeds will be imported for cultivation into the Europe.

The environmental risk for Europe is no greater than the risks associated with the import and processing of rice in commerce today.

A plan according to Annex VII for the import of genetically modified rice LLRICE62 has been prepared. This plan will provide for the general surveillance of unanticipated occurrence of adverse effects of the GMHP or its use on human health or the environment, which were not anticipated in the environmental risk assessment.