

**Finding of No Significant Impact**  
Animal and Plant Health Inspection Service  
Extension of Nonregulated Status to Rice Line LLRICE601

The Animal and Plant Health Inspection Service (APHIS) of the United States Department of Agriculture (USDA) received a petition for an extension of nonregulated status (APHIS number 06-234-1p) from Bayer CropScience of Research Triangle Park, NC for rice (*Oryza sativa* L.) designated as Liberty Link® Transformation Event LLRICE601. LLRICE601 has been genetically engineered for tolerance to the herbicide glufosinate. The request Bayer CropScience submitted seeks an extension of a determination of nonregulated status issued in response to APHIS petition number 98-329-01p for glufosinate-tolerant rice transformation events LLRICE06 and LLRICE62, the antecedent organism (see 64 FR 22595, published April 27, 1999, Docket No. 98-126-2).

A draft Environmental Assessment (EA) was prepared and submitted for public comment. APHIS received 15,871 comments on the subject EA during the designated 30-day public comment period, which ended October 10, 2006. APHIS' responses to the issues raised during the comment period are included as an attachment to this document.

APHIS considered three alternatives: Alternative A- No Action Alternative; Alternative B- Denial of Petition, Continuation as a Regulated Article; and Alternative C- Determination that LLRICE601 Plants are No Longer Regulated Articles. APHIS proposed Alternative C as its preferred alternative because of the similarity of LLRICE601 to the antecedent lines LLRICE62 and LLRICE06.

Based upon analysis described in the EA, APHIS has determined that the preferred alternative, to grant the petition, will not have a significant impact on the quality of the human environment because:

1. In assessing the risk of gene introgression from LLRICE601 into its sexually compatible relatives, APHIS considered two primary issues: a) the potential for gene flow and introgression; and b) the potential impact of introgression. Red rice is the only species likely to hybridize with glufosinate tolerant rice, and control practices are available to mitigate any effects of introgression should it occur. Therefore, there would be no impact related to outcrossing from deregulating this line (Alternative C). There would also be no impact from continuing to regulate the line (Alternatives A and B).
2. *O. sativa* does not persist in unmanaged ecosystems: Because it is only able to survive where rice is cultivated, and can be managed with existing cultural practices, there would be no weed impact from deregulating the genetically modified variety (Alternative C) and its subsequent release relative to the release of any conventional rice variety. There would also be no impact from continuing to regulate the line (Alternatives A and B).

3. There are unlikely to be any impacts on non-target organisms, including beneficial organisms and threatened or endangered species because the PAT protein is not known to have any toxic properties.

An analysis of federally-listed or proposed threatened and endangered species was conducted for the six major rice producing States (Arkansas, California, Louisiana, Mississippi, Missouri and Texas) and for three minor production States (Florida, Oklahoma, Tennessee) using the U.S. Fish and Wildlife database <http://ecos.fws.gov/ecos/index.do> and NatureServe database <http://www.natureserve.org/explorer/>. The engineered rice would not be expected to affect any of these species or other species that visit or inhabit rice fields since the PAT protein has been shown to be neither toxic nor allergenic (H rouet, et al., 2005). Likewise, based on the phenotypic similarity to conventional rice varieties in all respects other than glufosinate tolerance, APHIS concluded that LLRICE601 would not have any more impact on habitat of listed species than conventional rice. Under any of the alternatives, there would be no impact on non-target organisms or Federally-listed or proposed threatened and endangered species. EPA has reviewed submitted data, performed risk assessments, and approved the use of glufosinate herbicides on rice after careful consideration concluding that no "unreasonable harmful effects on endangered plants, other threatened or endangered species, or beneficial organisms" are expected from the use of glufosinate herbicides.

4. Analysis of available information indicates that LLRICE601 exhibits no traits that would cause increased weediness and that its unconfined cultivation should not lead to increased weediness of other cultivated rice or other sexually compatible relatives. LLRICE601 line exhibited no change in disease susceptibility, and it is unlikely to harm non-target organisms common to the agricultural ecosystem or threatened or endangered species recognized by the U.S. Fish and Wildlife Service. Based on this analysis, there is no apparent potential for significant impact to biodiversity. If APHIS chooses alternative A or B, there would also be no impact on biodiversity.

5. If LLRICE601 were to be grown commercially, the effect from introducing LLRICE601 into the environment on agricultural practices would be no different than for the deregulated lines. As the trait expressed is resistance to glufosinate and the level of resistance is similar to that of the other two lines, there should be no difference in impact on standard agricultural practices in rice cultivation and controlling volunteer rice.

6. APHIS analysis of data on agronomic performance, disease and insect susceptibility, and compositional profiles of LLRICE601 and its parent variety indicate no significant differences between the two that would be expected to cause either a direct or indirect plant pest effect on any raw or processed plant commodity from deregulation of LLRICE601. Similarly, there were no significant differences between LLRICE62 and LLRICE06 and their corresponding parent varieties. There would be no impacts on raw or processed agricultural commodities from deregulating the genetically modified variety (Alternative B). There would also be no impact from regulating the line (Alternative A).

7. The engineered plant is not different in any fitness characteristics from its parent that might increase its invasive potential.

8. None of the alternatives are expected to have significant human health or environmental effects.

Because APHIS has reached a finding of no significant impact, no Environmental Impact Statement will be prepared regarding this decision.

A handwritten signature in cursive script, appearing to read "Cindy Smith", written over a horizontal line.

Cindy Smith

Deputy Administrator

Biotechnology Regulatory Services

Animal and Plant Health Inspection Service

U.S. Department of Agriculture

Date: NOV 24 2006

**Attachment**  
**Finding of no significant impact**  
**Response to comments**  
**APHIS No. 06-234-01p**

In response to a notice published in the Federal Register (Docket No. APHIS-2006-0140, 71FR53076-53077) on September 8, 2006, APHIS received 15,871 comments on petition 06-234-01p and the related environmental assessment during the 30-day comment period.

354 respondents supported an extension of nonregulated status to LLRICE601. Supporters included rice growers, millers, or from related industries, rice industry groups, academia, a commodity company, a state government agency, and individuals. The majority of these respondents cited the similarity to the previously deregulated antecedent rice lines, the proven track record of safety of deregulated genetically modified organisms, reduction in pesticide uses, and a possible stabilizing effect on the rice market.

Comments received from respondents opposing deregulation of LLRICE601 were primarily from twelve public interest groups. 10,500 nearly identical comments were organized by one public interest group and 4796 nearly identical comments were organized by another public interest group. Other respondents were academic professionals, organic food producers, and individuals. Issues raised by those opposing deregulation include the failure to release confidential business information, the lack of independent research, the safety of the CaMV promoter and *bar* gene and the possible health effects for those affected with Celiac disease (severe gluten allergy), the contamination of organic crops by genetically engineered lines through crossing, the alleged increase in pesticide use, the rejection of U.S. crops exported to other countries, the effect of deregulating LLRICE601 after release, and the labeling of genetically modified foods.

In addition to the comments submitted to the docket, APHIS also received a Citizen's Petition from a public interest group that requested that all LibertyLink<sup>®</sup> Rice be listed as a plant pest. The Petitioner requested that the Citizen Petition be included in their public comment by reference. APHIS has included its response to the Citizen's Petition in this response to comments.

APHIS received many comments that suggested that the assessment of LLRICE601 was insufficient, that it was based on less data than other deregulations, and that the testing was not conducted over a long enough duration. One comment suggested, "The comparisons between LLRICE601 and the non-biotech variety are much less extensive than the standards for either USDA deregulation or for FDA review. The safety testing Bayer performed and submitted to USDA was substantially less than is usually accepted for deregulation." Another comment suggested, "Any similarity to other, US-approved, GM rice strains with regard to the parent strain and the DNA construct inserted is of no

scientific relevance to safety: the genome of LLRICE 601, and possibly the construct itself, will have been disrupted in a totally unique manner.” Another comment stated, “Bayer CropScience has not provided enough research to show that the rice lines designated as LLRICE601 is safe for human consumption without causing negative health effects such as allergies, regardless of its similarity to LLRICE62 and LLRICE06.”

APHIS regulates the introduction of GE organisms that may cause a plant pest risk. A person can petition APHIS to no longer regulate an organism by demonstrating that the organism does not present a plant pest risk.

APHIS has two processes by which a developer can petition for nonregulated status. The most commonly used process compares a regulated genetically engineered variety to an unmodified recipient organism. The applicant presents data on the similarities and differences between the transformed variety and the untransformed variety. Any differences are assessed as to whether those differences constitute a greater plant pest risk for the GE organism compared to the non-GE organism. Part of this assessment includes evaluating the interaction of the gene product with non-target organisms and the potential for the gene product to change the phenotype of the plant in a way that creates an increased plant pest risk.

The second process is an extension of nonregulated status which compares a regulated GE organism to a similar deregulated GE organism. Extensions of nonregulated status are a more streamlined process because the petitioner must only establish that the regulated and deregulated organisms are similar and therefore the regulated article presents no new issues that would require assessment under the full petition process. The extension process has been used fourteen other times since 1994.

APHIS is assessing LLRICE601 under the extension of nonregulated status process. The petitioner has supplied APHIS with information to support the assertion that LLRICE601 is similar to two previously deregulated rice lines, LLRICE62 and LLRICE06. The data in the petition includes information on the molecular, biochemical, and agronomic properties of LLRICE601. The petition contains adequate information to support the conclusion that LLRICE601 is similar to LLRICE62 and LLRICE06 and that it presents no new issues beyond those considered in the original petition. So while the petition may appear to have less data than is received for an evaluation of nonregulated status, it contains sufficient information to justify an extension of nonregulated status under 7 C.F.R. 340.6 (e). APHIS is not extending nonregulated status based on the similarity of the construct alone, as suggested by some comments. Molecular, biochemical and agronomic data were also considered in this assessment of similarity. The phenotypic data supplied in the petition supports the conclusion that LLRICE601 does not have unintended increased plant pest characteristics when compared to the antecedent lines.

Some comments suggest that because LLRICE601 is not identical to LLRICE62 or LLRICE06 that it should not be considered for an extension of nonregulated status. APHIS does not require that the lines or constructs are identical to meet the similarity requirement. Some of the issues raised include the difference in transformation method,

difference in length of promoter, and a different terminator sequence. APHIS has discussed all of these issues in its risk assessment, its preliminary determination and EA. None of these differences are significant or raise new issues that would warrant a full petition.

Many of the public comments expressed concerns with food safety issues. Some felt that testing was not conducted over a long enough period. Others felt that any GE product is unsafe for consumption. Still others were concerned that people with food allergies may be more sensitive to this rice and that genetically modified foods should be labeled. Food safety is outside of the regulatory authority of APHIS. If APHIS determines that an organism is no longer a regulated article under 7 C.F.R. 340, that determination is based on an assessment of plant pest risk, not food safety. Under the coordinated framework U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition (FDA/CFSAN) considers the safety of foods derived from biotechnology. On August 18, 2006 FDA released a statement regarding LLRICE601:

Bayer CropScience recently notified the U.S. Food and Drug Administration (FDA) and the U.S. Department of Agriculture Animal and Plant Health Inspection Service (APHIS) that trace amounts of a bioengineered variety of rice were detected in samples of commercial rice seed, and may have entered the food and feed supply in the United States. The bioengineered variety of rice, called LLRICE601, expresses the phosphinothricin-N-acetyltransferase (PAT) protein which provides tolerance to glufosinate-ammonium herbicide. This rice variety, not intended for commercialization, was not submitted to FDA for evaluation under the Agency's voluntary biotechnology consultation process. However, crops containing the PAT protein have previously been evaluated for safety by FDA on a number of occasions through the Agency's voluntary biotechnology consultation process. Bayer has informed the Agency that LLRICE601 is present in some samples of commercial rice seed at low levels. In addition, Bayer has provided information about the safety of the PAT protein, molecular characterization, and nutritional composition of grain from LLRICE601. Based on the available data and information, FDA has concluded that the presence of this bioengineered rice variety in the food and feed supply poses no food or feed safety concerns.  
(<http://www.cfsan.fda.gov/~lrd/biorice.html>)

One comment from a public interest group requested that APHIS not take any action on the petition for an extension of nonregulated status on LLRICE601 until Bayer CropScience has completed a consultation with the Food and Drug Administration under FDA's consultation procedures for foods derived from new plant varieties for LLRICE601. APHIS denies this request. LLRICE601 meets the criteria for an extension of nonregulated status under 7 C.F.R. 340. It is the obligation of the developer to seek all appropriate regulatory approvals.

Some comments confuse nonregulated status under 7 C.F.R. 340 with an approval for commercialization of a genetically engineered organism. Some comments suggest that because Bayer does not intend to commercialize LLRICE601, it should not be granted nonregulated status. Commercial markets have no bearing on the evaluation of a petition for extension of nonregulated status. Under APHIS regulations, a regulated article can be used for commercial purposes. The developer would only need to apply for the appropriate permits. Likewise a nonregulated article may never be commercialized. APHIS' decision is based only on plant pest issues not markets or commercial intent.

Several comments suggested that APHIS is considering "retroactive" deregulation of LLRICE601. Some of these comments also suggest that if APHIS grants an extension of nonregulated status to LLRICE601 that this action would shield Bayer from enforcement proceedings. APHIS disagrees with these comments. LLRICE601 has remained a regulated article throughout APHIS' consideration of the petition submitted by Bayer CropScience. Compliance issues and deregulation are handled separately. Even if LLRICE601 is deregulated, Bayer would still be responsible for any violations of APHIS regulations.

When APHIS considers a petition for nonregulated status or an extension of nonregulated status, we perform a thorough review of the submitted data, and the literature before making a determination. APHIS also considers any information brought forward during the public comment period that suggests that the subject of the petition is a greater plant pest risk than the untransformed variety. In the case of extensions of nonregulated status, APHIS bases its decision on similarity of the subject to a previously deregulated organism. APHIS is considering any information that suggests that LLRICE601 is not similar to LLRICE62 or LLRICE06. Only after this thorough, transparent, review does APHIS make a determination. Determinations of nonregulated status are not retroactive.

One public interest group and most of the nearly identical letters organized by that group, requested that APHIS not take action on the petition for an extension of nonregulated status for LLRICE601 until the compliance investigation is complete. They state that APHIS could not do a proper EA without knowing the extent of the unauthorized release of LLRICE601. APHIS disagrees with this comment and denies this request. APHIS has conducted a thorough evaluation of LLRICE601 and has determined that it is similar to LLRICE06 and LLRICE62. In analyzing the potential impacts on the environment, APHIS assumed that LLRICE601 may be grown commercially and, therefore, would be very widespread.

Similarly, APHIS' determination has no impact on the compliance investigation. BRS thoroughly evaluates all reports of compliance infractions. Serious infractions or cases in which BRS deems that a thorough investigation is warranted are referred to APHIS' Investigative and Enforcement Services (IES) for investigation. This case is currently being investigated by IES. To preserve the integrity of the investigations, information will only be posted after the investigation is complete and all enforcement actions have been finalized. The outcome of the investigation will be posted on

<http://www.aphis.usda.gov/brs/compliance.html> in order to document the inquiry and explain IES' findings.

Several comments stated that the amount of Confidential Business Information (CBI) in this petition makes it difficult to assess the scientific merit of the petition. Some stated that the claims are illegitimate and do not merit protection. The APHIS policy on CBI was published in 50 F.R. 38561. That policy requires information that is: 1) asserted to be a trade secret by the applicant; or 2) established by review to potentially cause substantial competitive harm, will be released only if required by statute or court order or otherwise required by law. Information of this nature is protected under the Freedom of Information Act (FOIA) (5 U.S.C. 552). Section (b)(4) of FOIA exempts from disclosure "trade secrets and commercial or financial information obtained from a person and privileged or confidential." 5 U.S.C. 552 (b)(4). Releasing this information can be a violation of the Trade Secrets Act (18 U.S.C. 1905).

APHIS has reviewed the CBI justification provided by the applicant and is maintaining the information in a way that is consistent with our policy. While CBI information is not available to the public, APHIS has thoroughly reviewed all of the information provided by the petitioner before making a decision.

A public interest group called for postponement of any decision on the deregulation of LLRICE601 until APHIS thoroughly reviews the merit of Bayer's CBI claims in accordance with its policy statement, releases for public review any information not deserving of CBI protection, and grants adequate time to offer informed comment on the petition. APHIS has reviewed the claims of CBI in accordance with its policy and has provided the public with the non-CBI content of the petition.

Many of the comments expressed concerns about social, economic, or trade issues. Many expressed concern that APHIS' decision may impact foreign and domestic markets for rice. Several comments suggested that APHIS' decision would have economic effects for rice producers, processors, and exporters. Some commenters believed that granting nonregulated status would improve rice markets. Others believed that granting nonregulated status would adversely impact trade and certain market sectors.

One comment suggested that, "[d]eregulation of LLRICE601 would likely have a calming effect on the marketplace," another stated that "economic harm would be avoided by approving the LLRICE601 for human food use."

When APHIS considers a petition for nonregulated status or an extension of nonregulated status, it considers the potential increased plant pest risk of the subject of the petition. The decision is based on scientific evidence. Market, economic or trade issues are not considered in an assessment of plant pest risk.

One comment from a public interest group stated that:

The agency has failed to address a number of other socioeconomic impacts that must be addressed as part of the National Environmental Protection Act (NEPA) process. Indeed, the Council on Environmental Quality (CEQ) regulations implementing NEPA state that such impacts must be analyzed. Among the issues that need to be addressed include: (1) impact of LLRICE601 on U.S. rice exports and export of U.S. products using rice derived from rice contaminated with LLRICE601; and (2) the impact of allowing LLRICE601 that is subject to utility patent protection will affect farmers;

They go on to state:

Federal courts have also upheld that NEPA requires, where economic analysis forms the basis of choosing among alternatives, that the analysis not be misleading, biased or incomplete. Seattle Audubon Society v. Lyons, 871 F.Supp. 1291 (W.D. Wash., 1994).

APHIS did not address these issues in the EA because they are not environmental issues, but are essentially market issues. Additionally, the commenter misinterprets CEQ's NEPA implementing regulations because it confuses the requirements of an EIS with those of an EA. Since the issues raised by the comment are related to potential market effects and are not environmental, they go well beyond the scope of this EA. CEQ's own guidelines suggest that:

Since the EA is a concise document, it should not contain long descriptions or detailed data which the agency may have gathered. Rather, it should contain a brief discussion of the need for the proposal, alternatives to the proposal, the environmental impacts of the proposed action and alternatives, and a list of agencies and persons consulted. Section 1508.9(b). <http://ceq.eh.doe.gov/nepa/regs/40/30-40.HTM#36>.

With respect to this comment, USDA believes that all methods of agricultural production (conventional, organic, or the use of genetically engineered varieties) can provide benefits to the environment, consumers, and the agricultural economy. The role of Biotechnology Regulatory Services within APHIS is to provide regulatory oversight that allows for the safe development and use of genetically engineered organisms. Once a new biotech variety has been granted nonregulated status by APHIS, any decisions to produce or market that product are made by the technology providers and producers and are driven by market demand. USDA encourages the developers of new biotech varieties to seek regulatory approvals for these new products in our major export markets at the same time nonregulated status is sought within the US, to help prevent loss of markets that could result from unapproved genetically engineered products entering the export channels. The USDA Grain Inspectors, Packers, and Stockyard Administration (GIPSA) announced in August 2002 that they will be developing voluntary testing and process

verification programs to facilitate the marketing of agricultural products such as non-genetically engineered varieties.

Many comments expressed a general disapproval of all GE crops. Many of these comments expressed a fear of the technology. Others felt that the development of GE crops was not ethical. Many other comments supported the use of GE crops and the deregulation of this one.

APHIS makes a determination on a request for nonregulated status based on scientific data. The regulations in 7 C.F.R. 340.6 describe the process that APHIS uses. The determination is based on whether the regulated article is more of a plant pest risk than the non-GE variety, or in an extension of nonregulated status, is similar to the antecedent organism. Many people have differing opinions about the overall safety of GE organisms. Some people believe that they should not be regulated, while others believe that they should not be developed. Deregulation is not based on opinions or the number of people who comment during the public comment period. Deregulation is only based on scientific data. APHIS considers scientific data provided by the applicant, published in scientific journals, and provided by interested parties during the public comment period.

Many of the public comments expressed concerns with food safety issues. Some felt that testing was not conducted over a long enough period of time. Others felt that any GE product is unsafe for consumption. Still others were concerned that people with food allergies may be more sensitive to this rice. Some comments were concerned with potential allergenicity of LLRICE601. A few comments expressed a concern that LLRICE601 may adversely affect people with Celiac disease. Celiac disease, also known as Gluten-Sensitive Enteropathy or Celiac Sprue, is autoimmune gastrointestinal disorder that results in damage to the mucosa of the small intestine after ingestion of wheat gluten or related rye and barley proteins (OMIM 212750, <http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=212750>). FDA addressed this issue in LLRICE62 and LLRICE06 as follows:

Rice is often used as a dietary substitute for wheat in patients suffering from celiac disease. Celiac disease is an intestinal disease that manifests itself as an atrophy of the mucosae of the small intestine, resulting in malabsorption of food. A group of proteins known as prolamines are responsible for the disease. Prolamines are part of the Osborne fraction. The Osborne fraction is composed of albumin, globulin, glutelin, and prolamins proteins. Rice contains the Osborne fraction but in much lower quantities than does wheat, rye, or barley.

The notifier did not find significant differences in the content of these proteins in brown rice samples of LLRICE06 and LLRICE62 as compared to their respective non-transgenic counterparts. (<http://www.cfsan.fda.gov/~rdb/bnfm063.html>)

LLRICE601 has not undergone a full consultation with FDA nor was it specifically tested for an increase in prolamines. Commenters did not offer support to suggest that there would be a change in the levels of these proteins.

APHIS also received a comment from a Professor of Food and Beverage Management who is also a Registered Dietician. The comment explains the relationship between the storage protein gliadin and Celiac Disease. It also states that it is unlikely that this line of rice would have any effect on patients with Celiac Disease. A second comment received from a Professor of Food Science and Technology who specializes in Food Allergy research also stated that, “the data that is readily available supports the conclusion that there is no increased risk of allergy or Celiac disease associated with LLRICE601, compared to non-GM rice.” Food safety is outside of the regulatory authority of APHIS. Under the coordinated framework U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition (FDA/CFSAN) considers the safety of foods derived from biotechnology.

Several of the comments expressed concerns about the unintended effects of genetic engineering. One such comment stated that, “serious health problems [were] encountered by laboratory animals fed GM food.” The comment offers no support for the statement. Many comments related unintended effects to concerns for food safety or plant pest risks. Some of the comments suggested that these effect may be plant pest issues or have environmental effects not fully analyzed by APHIS. APHIS evaluates potential unintended effects that may result in plant pest risk issues through its assessment of molecular and agronomic data. There are no indications that there are any unintended plant pest characteristics in LLRICE601.

One commenter was concerned about the potential insertion of a portion of an additional copy of the CaMV 35S promoter. The comments suggest that this may create an unintended effect by expressing a gene. APHIS considered this possibility in its risk assessment of LLRICE601 (see page 19 of the EA, [http://www.aphis.usda.gov/brs/aphisdocs/06\\_23401p\\_ea.pdf](http://www.aphis.usda.gov/brs/aphisdocs/06_23401p_ea.pdf)). While APHIS agrees that there is a small possibility that the CaMV 35S sequence that potentially inserted may alter the expression of a native gene or pseudogene, the phenotypic data revealed no apparent difference between LLRICE601 and the parent variety. If there are any changes in gene expression, they do not appear to pose a plant pest risk. Another comment suggested that since LLRICE601 may contain a portion of the vector backbone, that it may contain the antibiotic resistant selectable marker. While the specific experimental results are CBI, APHIS has examined the data and has concluded that LLRICE601 does not contain any part of the antibiotic resistance gene.

A few comments also mentioned that APHIS failed to investigate potential pleiotropic or unintended effects. One comment suggests, “In particular, unintended effects may differ between each transformation event, and therefore, unintended effects should be as thoroughly examined in an extension petition as in a typical non-extension petition.” Two of the cited unintended effects are LLRICE601’s short stature compared with the parent line and LLRICE601’s moderate level of seed shattering compared to very low

levels in other LibertyLink<sup>®</sup> rice lines and their progenitors. The progenitor to LLRICE601, Cocodrie, shatters more than the other LibertyLink progenitors. The “seed shatter result” observed in LLRICE601 is not an unintended effect; it is due to the genetic background of the progenitor line. APHIS reviewed the agronomic data in Bayer’s petition and agreed with the conclusion that LLRICE601 is not different from its parental variety in any characteristic measured including height or seed shatter. Neither characteristic raises any new issues that would warrant a full petition for nonregulated status.

Another commenter was concerned that APHIS failed “to require analysis of LLRICE601 seeds for three known antinutrients found in rice: phytic acid, lectin, and trypsin inhibitor.” APHIS does not have authority to regulate on the basis of food safety and as such does not require data on antinutrients. In addition, Bayer has provided information to FDA about the safety of the PAT protein and molecular characterization of grain from LLRICE601. Based on the available data and information, FDA has concluded that the presence of this bioengineered rice variety in the food and feed supply poses no food or feed safety concerns.

Another concern is that APHIS “failed to request data and investigate potential changes in LLRICE601’s susceptibility to insect pests”. Bayer made observations on the susceptibility of LLRICE601 to water weevil, stem borer, and stinkbug, the principal insect pests of rice. No differences were observed between LLRICE601 and non-transgenic controls planted at the test sites.

A few comments suggested that glufosinate resistance in red rice would increase the weediness of this rice weed and make it more of a plant pest. APHIS disagrees with this suggestion. The *bar* gene does not confer any fitness advantage to red rice other than resistance to glufosinate herbicide. If the *bar* gene would introgress into red rice and make red rice glufosinate tolerant, the red rice would not become weedier. It would simply mean that glufosinate would be less effective to control red rice. Glufosinate is not currently used to control weeds in rice.

One commenter suggested that glufosinate may be teratogenic. This comment is related to the use of the herbicide glufosinate, not the potential plant pest risk of LLRICE601. EPA considers these issues under its authority. APHIS defers to EPA on this issue. EPA has previously responded to the comment that glufosinate causes reproductive and developmental toxicity as follows:

The potential for glufosinate ammonium to cause developmental or reproductive effects due to exposure (male or female) has been evaluated in acceptable guideline studies in rats and rabbits. Based on these studies, glufosinate ammonium is not teratogenic in rats and rabbits (68 F.R. 55833-55849).

A public interest group claimed that, “Assertions in the Environmental Assessment prepared by USDA-APHIS are in conflict with pronouncements made by the USDA

National Organics Program (NOP).” The comment cites the following statement in the USDA APHIS’ environmental assessment, “the presence of a detectable residue of a product of excluded methods alone does not necessarily constitute a violation of the National Organic Program” and compares this with a statement from the National Organics program, “In the event that a producer or handler tests rice and finds presence of genetically engineered materials, the rice will not be considered organic.” To address the concerns of the public interest group, the two statements in question were submitted to the Agricultural Marketing Service (AMS), the USDA agency that administers the National Organics Program. In response, AMS provided the following statement of clarification:

The statements above are consistent. The statement from the National Organic Program (NOP) was referring to the issue of resolving contractual settlements in the market between buyers and sellers if testing occurs. Excluded methods are prohibited; however, the NOP regulations make no claim to be GM-free. When all regulations have been followed, the detection of adventitious presence does not necessarily result in loss of organic status for a product—that issue is left to the buyer and seller to resolve in the market. The statement referred to was an attempt to clarify that, if testing were to be conducted and an excluded method were detected, there would be a basis in the NOP regulations for a buyer and seller to revisit their contract. It was not intended and, in fact, does not change the reality that this type of situation is left for resolution in the market.

The same public interest group also claimed, “The basis under which USDA/APHIS suggested that LLRICE601 will not have a significant impact on organic farming is incorrect.” The environmental assessment does not state that LLRICE601 will not have a significant impact on organic farming, but rather it assesses the impact of APHIS alternative actions in responding to the request to extend nonregulated status. Specifically, it concludes that “granting the extension of nonregulated status (alternative C) will have no significant impact on organic farming.” Granting or not granting the extension will not affect the organic status of rice in which genetically engineered materials are detected.

One commenter suggested that “The USDA should require companies to provide the department with the molecular probes needed to detect genes and/or gene products from GE food/feed crops field tested in the United States.” This issue is not specifically related to the LLRICE601 petition or the Environmental Assessment. However, APHIS does consider this to be an important issue. While APHIS does not believe that this is necessary for every field trial, we are currently discussing whether and under what circumstances APHIS should require that validated testing methods be made available.

Several comments raised issues related to production methods for rice and the change in use of herbicides on rice. Many of the comments that support the deregulation of LLRICE601 suggest that this “will provide an alternative production method that rice

producers need and should result in the decreased use of herbicides.” Others who oppose granting nonregulated status suggest that it may increase the use of pesticides. Many factors will contribute to the production methods that are used and the pesticides that may be applied to LLRICE601 should it be grown commercially. As stated in the EA (pg.7) crop rotation, irrigation management and herbicides are used to control weeds in rice and no single weed management strategy is successful for control. Herbicide tolerant varieties of rice (Clearfield®) are already available.

One comment “recommends USDA/ APHIS enact policies that recognize that crops which are historically central to the economy and world view of Native people should be considered exempt from transgenic experimentation in the open environment.” The request is made in reference to wild rice. Wild rice species (*Zizania sp.*) are different species from the subject of the petition (*Oryza sativa*). APHIS has not had any permit or notifications, acknowledged, issued or pending for wild rice. The request is outside the scope of the petition and the EA for LLRICE601.

As mentioned above, a Citizen Petition ([http://www.centerforfoodsafety.org/pubs/LLRice\\_Petition\\_9.13.06.pdf](http://www.centerforfoodsafety.org/pubs/LLRice_Petition_9.13.06.pdf)) was submitted to the USDA and the petitioner requested that the petition be included in their public comment by reference.

The Citizen Petition requested the Secretary take the following actions:

1. Determine that LibertyLink® rice is a plant pest under the Plant Protection Act §7711
2. Add LibertyLink® rice to the list of organisms that are plant pests
3. Determine that LibertyLink® rice is a regulated article and restrict its introduction, dissemination, interstate movement, and conveyance under 7 CFR §340.

The Citizen Petition was accompanied by 75 supporting articles listed in an index at the following link: [http://www.centerforfoodsafety.org/pubs/LLRice\\_Appendix9.13.06.pdf](http://www.centerforfoodsafety.org/pubs/LLRice_Appendix9.13.06.pdf). APHIS thoroughly reviewed the Citizen’s Petition and the seventy five supporting documents prior to any agency action or decision on LLRICE601. The Citizen Petition requested that APHIS consider five principal arguments which, in the view of the Petitioner, make the prior determination on LLRICE06 and LLRICE62 “erroneous” in light of “new evidence based on sound science that demonstrates that LibertyLink® rice will injure and damage plants, crops, and the environment.”

**The first argument made in the Citizen Petition is the claim that LibertyLink® rice will increase the weediness of red rice and other weeds, either by gene flow of the LibertyLink® resistance gene to red rice, or by direct selection of glufosinate resistant weeds in response to glufosinate use.**

APHIS disagrees with this comment because the *bar* gene does not confer any fitness advantage to red rice other than resistance to glufosinate herbicide. In the worst case scenario discussed by the Petitioner, where the *bar* gene would introgress into red rice, glufosinate tolerant red rice would not become weedier. It would simply mean that

glufosinate would be less effective to control red rice. As glufosinate is not currently used to control weeds in rice, it would mean that red rice would not be any weedier than it is today. The same chemical, physical, and mechanical control practices used today would be available to control red rice and any other weeds that would develop glufosinate tolerance.

Even if a glufosinate tolerant red rice population develops, all red rice will not become glufosinate tolerant and glufosinate and other control practices are still likely to be effective control methods for LibertyLink<sup>®</sup> rice. For example Powles et al. (Petitioner ref #28) note that *Lolium rigidum*, a plant that has developed resistance to multiple herbicides including glyphosate, is still effectively controlled by glyphosate. According to Powles et al. (Petitioner ref #28):

*Lolium rigidum* is a major weed of cropping throughout southern Australia and thousands of populations have evolved herbicide resistance. Multiple resistance is also evident in *L. rigidum* and extends across many herbicide chemistries. The Integrated Weed Management (IWM) strategies employed to manage herbicide-resistant *L. rigidum* include various combinations of factors including pasture and crop rotations, variation in seeding date, use of nonselective herbicides, high crop seeding rates, vigorous crop growth, and capture of weed seed in the harvest operation. It should be noted that despite resistance, herbicides remain an integral component of IWM strategies for *L. rigidum* control in cropping systems in Australia. Thus, even when faced with multiple resistances, herbicides have remained pivotal to the success of an IWM strategy.

The Petitioner suggests that introgression of glufosinate tolerance into red rice would develop in 3 to 8 years based on a 2002 modeling study (Petitioner ref #13). APHIS notes that introgression of glufosinate tolerance into red rice does not make red rice weedier and therefore the rate of introgression is not a factor in the determination of whether LibertyLink<sup>®</sup> is a plant pest. Moreover the analysis in ref #13 is fundamentally flawed because it assumes that glufosinate control would only be 75%, a control so poor that the product would be a failure and substantially inferior to what has been observed in field trials. Studies examining the impact of weeds on rice yield have determined that 5 and 20 red rice plants/m<sup>2</sup> reduce yield by 40 and 60%, respectively (Fischer and Ramirez, 1993). At a 75% control rate, weeds would be present at a density of around 33-45 plants/ m<sup>2</sup> assuming the optimal density of rice is 135-180 plants/m<sup>2</sup> (<http://deltafarmpress.com/news/050323-rice-seeding/>.) or close to twice the density that reduced yield by 60%. In U.S. trials, control is estimated to be 94-100% with two glufosinate applications at a rate of 0.4 g ai/acre (Wheeler and Baldwin, 1997). With effective control, very little red rice would be in proximity to the LibertyLink<sup>®</sup> rice so the time needed for introgression would be considerably more than estimated in the model.

The Petitioner comments that USDA incorrectly estimated the plant pest risk because at the time of the original EA, USDA knew little about the fitness of red rice with introgressed glufosinate tolerance. They raise issues such as once the *bar* gene has

introgressed, the resulting plants will have varying flowering times and plant height, the resulting plants may mimic cultivated rice and be difficult to detect, red rice may acquire multiple herbicide resistances, red rice has dormancy and shatters and therefore the trait may persist.

APHIS did not underestimate the plant pest risk because it considered the impact assuming the *bar* gene did in fact introgress into red rice resulting in an herbicide tolerant form of the plant possessing all the weedy characteristics of red rice. As described above, APHIS based its estimate of the plant pest risk on the fact that if the gene introgressed into red rice, other control methods would still be available to mitigate any increased plant pest risk that might arise. Even though APHIS believes introgression rates will be low because rice pollen is heavy, remains viable for only about 10 minutes after shed, and is only shed about 1 hour per day, any differences in the actual rate of introgression are largely unimportant.

Petitioners comment that if any red rice plants develop tolerance to glufosinate, LibertyLink<sup>®</sup> rice should continue to be regulated. They state:

Gene introgression from LibertyLink<sup>®</sup> rice into red rice will be permanent and will increase its weediness by giving red rice a fitness advantage in an agricultural setting. Since red rice is already a serious weed, USDA should regulate LibertyLink<sup>®</sup> rice to prevent escalation of the injury caused by red rice.

APHIS disagrees with this view. LibertyLink<sup>®</sup> rice offers a means of herbicide control that does not currently exist. Glufosinate cannot now be used to control red rice because it also kills cultivated rice. The availability of LibertyLink<sup>®</sup> rice lines would enable glufosinate to be used on rice for red rice control. This new means of herbicide control may offer benefits in the form of improved weed control at lower costs with less damage to the environment (Gianessi *et al.* 2002). If gene introgression from LibertyLink<sup>®</sup> rice into red rice were to occur, there would be no escalation of injury as the glufosinate resistance trait does not confer a fitness advantage and glufosinate is not currently used to control red rice. In the absence of glufosinate use, red rice with the *bar* gene could be managed by the same practices used to manage red rice lacking the trait. If the effectiveness of glufosinate to control red rice diminishes over time due to introgression of the *bar* gene into red rice, the effect is one of lost benefits from the technology and not an incremental injury. The worst case scenario is that 100% of the red rice would become tolerant to glufosinate. However, this situation is highly improbable because integrated weed management strategies are often employed to effectively manage the development of resistance. If the worst case scenario were to occur, this situation would be no worse than the *status quo* sought by the Petitioner where LibertyLink<sup>®</sup> rice would continue to be regulated and glufosinate would not be available to control red rice in cultivated rice crops.

Petitioner raises the concern that “resistant management plans will be insufficient to prevent herbicide-tolerance in red rice”. They conclude that the recommended control

rate of two glufosinate applications will not be followed. APHIS does not have the authority to mandate stewardship programs and did not consider stewardship in the determination for LLRICE06 and LLRICE62.

APHIS notes that growers often resort to integrated weed management programs (IWM) to better manage their cropping systems. The recommended strategies for red rice control include: (<http://plu.tamu.edu/RiceContestStudyGuide/2004/Red%20Rice.pdf>):

- preplant-incorporated herbicide applications
- post-planting herbicide applications (for Clearfield® rice, a non genetically engineered rice variety tolerant to imidazolinone herbicides)
- continuous or pinpoint flooding
- use of weed free seed
- diligent cleaning of farm equipment
- crop rotations.

Introduction of LibertyLink® rice into IWM programs, should it ever occur, would represent a minor change to current practice as herbicide tolerant rice is currently in use and widely adopted (Davis, 2006). The availability of LibertyLink® rice will allow the two herbicide tolerant varieties (Clearfield® and LibertyLink® rice) to be interchanged in a rotation with other crops such as soybean to alter the selection pressure. Adoption of the Clearfield® and LibertyLink® rice systems also results in the adoption of the IWM practice to use weed free seeds. One of the big problems in managing red rice is the practice of saving seed or planting seed purchased from farmers that does not have to meet state regulations (Petitioner ref # 17) and which can result in the perpetuation of red rice contamination of seed. With either herbicide tolerant rice system, farmers must purchase certified seed each year and certified seed may not contain any red rice (Petitioner ref # 17). Prudent stewardship requires a multiplicity of control methods and glufosinate use on LibertyLink® rice represents a new control method to add to the list of integrated weed management options.

The Petitioner also commented that glufosinate use on LibertyLink® rice will lead to the direct selection of glufosinate resistant weeds and that these weeds will become a serious pest problem and injure plants. The Petitioner requests that USDA look at the potential for glufosinate resistance to develop in major rice weeds including barnyard grass, smallflower umbrellaplant, redstem, ducksalad, watergrass, and rice weeds. APHIS acknowledges that there is a potential risk for pest resistance to emerge in response to any management practice that imposes a selection. For example, weeds will develop resistance to herbicides applied to conventional rice, as has barnyard grass and junglerice to propanil, the most widely used herbicide on rice (Lopez-Martinez *et al.* 2001). LibertyLink® rice does not pose unique risks for the evolution of herbicide resistant weeds when compared to conventional rice varieties and therefore cannot be considered to be a plant pest on this basis. As mentioned above, there are other means to control weeds in rice. Even if weeds develop resistance to an herbicide, that herbicide may still be useful in an integrated weed management program.

**The Petitioner’s second argument for why LibertyLink® rice is a plant pest is the claim that LibertyLink® rice will damage agricultural commodities because it will ultimately commingle with commodity rice.** The Petitioner refers to the recent detection of this rice event in commodity rice and the consequential effect on domestic and international markets. Although the commingling has resulted in commodity rice losing value, the commingling has not damaged the agricultural commodity. The loss in value to exports or organic products brought about by commingling is a marketing issue and is not based on the safety of the product. The likelihood of LibertyLink® rice commingling with commodity rice is no greater than any other variety. APHIS acknowledges that some markets have not accepted LibertyLink® rice. However market acceptance is not a plant pest issue and does not effect APHIS’ decision to grant nonregulated status.

The Petitioner misunderstands what is meant by damage in the context of the Federal Plant Pest Act, which has been superseded by the Plant Protection Act (7 U.S.C. 7758). They refer to Senate Report 189, Federal Plant Pest Act (Mar. 26, 1957), “(noting that one of the aims that the Act sought to cure was the spread of witchweed, a plant that greatly reduces the yield, making the crop uneconomic).” From this example, the Petitioner mistakenly infers that the Act gives APHIS the authority to intervene over market issues which may cause “economic harm.”

APHIS’ regulations at 7CFR 340 are based on the authority to control plant pests. Plant pest is defined as “a living organism that can directly or indirectly injure, cause damage to, or cause disease in any plant or plant product.” Therefore, this definition does not provide authority to regulate because of market effects.

LibertyLink® rice is indistinguishable from conventional rice with the exception of its resistance to glufosinate. It does not directly or indirectly injure, cause damage to, or cause disease to other rice varieties and is, therefore, not a plant pest. The impact of LibertyLink® rice on the value of rice is driven by market issues, and this impact can change by a difference in market acceptance without any alteration to the properties of LibertyLink® rice.

On a related topic, the Petitioner comments that LibertyLink® rice will damage the purity of seed stocks. Comments include the following:

“introduction of LibertyLink® rice will make seed stocks vulnerable to genetic contamination thus damaging rice seed.”

“maintaining breeder and foundation seed stocks free from genetically engineered traits is critical to ensure that farmers may still obtain non-genetically engineered rice varieties”

“LibertyLink® rice is a plant pest because it can contaminate rice seed and damage the genetic purity of breeder and foundation stocks.”

LibertyLink<sup>®</sup> rice varieties pose no unique threat to the purity of seed stocks. Any seed stock has vulnerabilities to commingling with other varieties, whether they be GM or conventional. APHIS notes that the threshold for how much off-types or commingling of varieties are permissible in foundation and certified seed stocks are defined in the Association of Official Seed Certifying Agencies (AOSCA) standards (Association of Official Seed Certifying Agencies, 2001). For rice, foundation seed may have no more than 0.05% seed of other varieties or off-types while certified seed can have no more than 0.2%. The Petitioner has the mistaken notion that there are different AOSCA standards for GM crops that have nonregulated status and non GM crops. Petitioners cite articles such as Mellon and Rissler (Petitioner reference #43) which “warn about the transgenic contamination of the traditional seed supply,” when, in fact, the cases reported fall within the standards for certified seed. The potential to commingle is not a viable reason to consider LibertyLink<sup>®</sup> rice a plant pest.

**The Petitioners third argument for why LibertyLink rice is a plant pest is the claim that herbicide tolerant rice will lead to increased glufosinate use and thereby harm threatened and endangered species or beneficial organisms.**

In making their arguments, the Petitioner raises several issues that are outside APHIS’ regulatory authority. The response to this petition is based on APHIS’ authority and only those issues that are relevant to APHIS’ authority. APHIS has a congressionally mandated authority to determine whether a genetically engineered organism is a plant pest as codified in the Plant Protection Act. However, Petitioners raise a number of issues related to glufosinate use on LibertyLink<sup>®</sup> rice that are not relevant to the Plant Protection Act. As outlined below, the issues related to glufosinate use are within the jurisdiction of the EPA.

**EPA, and not USDA, has authority to register pesticides and set tolerances.**

EPA receives its authority to register pesticides under the [Federal Insecticide, Fungicide, and Rodenticide Act \(FIFRA\)](#) and authority to set tolerances under the Federal Food, Drug, and Cosmetic Act (FFDCA). Pesticide registration is the process through which EPA examines the ingredients of a pesticide; the site or crop on which it is to be used; the amount, frequency and timing of its use; and storage and disposal practices (<http://www.epa.gov/pesticides/regulating/registering/index.htm>)

**EPA evaluates the pesticide to ensure that it will not have unreasonable adverse effects on humans, the environment and non-target species.**

To make such determinations, EPA requires more than 100 different scientific studies and tests from applicants (<http://www.epa.gov/pesticides/regulating/>). Many plant and wildlife species can be found near or in cities, agricultural fields, and recreational areas. Before allowing a pesticide product to be sold on the market, EPA ensures that the pesticide will not pose any unreasonable risks to wildlife and the environment. EPA does this by evaluating data submitted in support of registration regarding the potential hazard that a pesticide may pose to non-target fish and wildlife species. In considering whether to register a pesticide, EPA conducts ecological risk assessments to determine what risks are posed by a pesticide and whether changes to the use or proposed use are necessary to

protect the environment. A pesticide cannot be legally used if it has not been registered with EPA's Office of Pesticide Programs.

### **EPA Sets Tolerances To Ensure Food Safety**

(<http://www.epa.gov/pesticides/factsheets/stprf.htm>)

Before allowing the use of a pesticide on food crops, EPA sets a tolerance, or maximum residue limit, which is the amount of pesticide residue, allowed remaining in or on each treated food commodity. The tolerance is the residue level that triggers enforcement actions. That is, if residues are found above that level, the commodity will be subject to seizure by the government. In setting the tolerance, EPA must make a safety finding that the pesticide can be used with "reasonable certainty of no harm." To make this finding, EPA considers:

- the toxicity of the pesticide and its break-down products
- how much of the pesticide is applied and how often
- how much of the pesticide (i.e., the residue) remains in or on food by the time it is marketed and prepared

EPA ensures that the tolerance selected will be safe. §408(b)(2)(A)(ii) of the FFDCA defines "safe" to mean that:

There is a reasonable certainty that no harm will result from aggregate exposure to the pesticide chemical residue, including all anticipated dietary exposures and all other exposures for which there is reliable information.

### **Tolerance Setting Requires Numerous Scientific Studies**

Pesticide companies, or registrants, must submit a wide variety of scientific studies for review before EPA will set a tolerance. The data are designed to identify possible harmful effects the chemical could have on humans (its toxicity), the amount of the chemical (or breakdown products) likely to remain in or on food, and other possible sources of exposures to the pesticide (e.g., through use in homes or other places). All of this information is used in EPA's risk assessment process. The risk assessment includes consideration of the amounts and types of food people eat and how widely the pesticide is used (that is, how much of the crop is actually treated with the pesticide), as well as chemistry, toxicity, and exposure information. EPA also uses data from USDA on what foods people eat and the quantity they eat, collected through the Pesticide Data Program.

The following specific issues were raised about glufosinate use that are outside the scope of APHIS' authority and are irrelevant to APHIS' decision regarding whether LibertyLink<sup>®</sup> rice is a plant pest:

- 1) "LibertyLink<sup>®</sup> rice will injure non-target organisms because its dissemination will drastically increase the amount of herbicide used in rice growing areas." Furthermore, "USDA cannot rely on EPA's pesticide registration to prevent

- injury because it is the farming system associated with LibertyLink<sup>®</sup> rice itself that will increase the overall amount of glufosinate use.”
- 2) That increased use of the pesticide will harm non-targets and threatened and endangered species, such as clam and oyster larvae, shrimp, water fleas, some freshwater fish, mallard ducks, and predatory arthropods.
  - 3) That the surfactant in glufosinate may have adverse effects on amphibians.
  - 4) That glufosinate use will change the soil biota and thereby decrease soil fertility, decomposition of organic matter, nitrogen availability, water retention, and prevalence of soil borne diseases.
  - 5) That genetically engineered plants metabolize glufosinate differently than conventional plants and this will lead to higher human exposures to glufosinate and its metabolites. (Note this same question was raised to EPA and has been addressed publicly (U.S. EPA, 2003).
  - 6) That glufosinate is a teratogen (Note this same question was raised to EPA and has been addressed publicly (U.S. EPA, 2003).

USDA disputes the Petitioner’s assertion that APHIS cannot rely on EPA’s pesticide registration to prevent injury to threatened and endangered species and or non target species in rice growing areas. In the registration of Liberty<sup>®</sup> and Rely<sup>®</sup> herbicides on rice, estimated environmental exposures were calculated based on conservative models that are not likely to be exceeded based on a maximum herbicide application of 0.44 lbs a.i./A twice during the growing season. During the registration process EPA assessed the potential effects of both the active ingredient and the formulation (including surfactants) to aquatic animals using fish as surrogates for amphibians. In using surrogates, EPA incorporates uncertainty factors to build in a conservative estimate in the risk assessment. EPA has reviewed the submitted data, performed risk assessments, and approved the use of Liberty<sup>®</sup> on rice after careful considerations concluding that no "unreasonable harmful effects on endangered plants, other threatened or endangered species, or beneficial organisms" are expected from the use of Liberty<sup>®</sup>.

Glufosinate use on LibertyLink<sup>®</sup> rice was granted Reduced Risk Status by the EPA on September 30, 2003. (<http://www.epa.gov/opprd001/workplan/completionsportrait.pdf>). In PR Notice 97-3 ([http://www.epa.gov/oppmsd1/PR\\_Notices/pr97-3.html](http://www.epa.gov/oppmsd1/PR_Notices/pr97-3.html)) EPA states that reduced risk status is granted to registrations for pesticides that are expected to accomplish one or more of the following:

- (i) Reduce the risks of pesticides to human health.
- (ii) Reduce the risks of pesticides to nontarget organisms.
- (iii) Reduce the potential for contamination of groundwater, surface water or other valued environmental resources.
- (iv) Broaden the adoption of integrated pest management strategies, or make such strategies more available or more effective.

In the case of glyphosate, another broad spectrum herbicide which also has reduced risk status, the adoption of herbicide-tolerant crops lead to the substitution of glyphosate for previously used herbicides which were more toxic (Fernandez-Cornejo and McBride 2002). Expectations are that adoption of LLRICE will similarly reduce the use of more

toxic pesticides and lead to the adoption of environmentally beneficial management practices (Gianessi *et al.* 2002).

The Petitioner commented that glufosinate use may have indirect effects on migratory birds because glufosinate will be so effective in controlling weed species that insects that depend on these weed species, and in turn the birds that feed on these insects, will be adversely impacted. APHIS considers this concern to be without merit. First, this indirect effect is no different than for any weed control program. Second, two of the major weeds of rice fields that are targets for control by glufosinate, red rice and sprangletop, are on the Federal Noxious Weed List and several others, such as barnyardgrass, nutsedge, alligator weed, and morning glory are invasive species and on State Noxious Weed Lists (<http://plants.usda.gov/java/noxComposite?sort=comname>). Four of these weeds are subject to either Federal or State Quarantines. A fundamental aim of the Plant Protection Act is to detect, control, eradicate, suppress, prevent, or retard the spread of plant pests or noxious weeds.” APHIS notes that the more effective weed control afforded by LibertyLink<sup>®</sup> rice/glufosinate against these plant pests and weeds is in direct accord with the aims of the Plant Protection Act. The idea that LibertyLink<sup>®</sup> rice, a form of cultivated rice, should be listed as a plant pest while Federal and State noxious weeds should be allowed to grow in rice fields to support wild life is a conflation of the Plant Protection Act.

**The Petitioner’s fourth argument for why LibertyLink<sup>®</sup> rice is a plant pest is the claim that LibertyLink<sup>®</sup> rice varieties are more likely to become weeds than conventionally bred varieties.** APHIS does not see any basis for this claim. The *bar* gene has been thoroughly evaluated and does not confer fitness properties besides glufosinate tolerance. Rice is not weedy by nature. It requires very specialized conditions for growth. Even weedy red rice is not a problem weed in other agronomic settings. Rice is very easy to control, either by altering the cultivation conditions, by physical means, or by chemical means. Rice is usually rotated with soybeans and most soybeans are resistant to glyphosate whereas LibertyLink<sup>®</sup> rice is not. APHIS does not anticipate there will be any difficulty for farmers to control LibertyLink<sup>®</sup> rice in rotation crops.

**Petitioner’s fifth argument for why LibertyLink<sup>®</sup> rice is a plant pest is the claim that LibertyLink<sup>®</sup> rice may exhibit unpredicted properties that could bear on its plant pest traits including pathogenic properties. For example, the Petitioner’s state that:**

Recent studies show that unintended characteristics may arise from genetic engineering because genes and proteins change and produce unexpected adverse effects. Insertion of DNA sequences can modify, interrupt, or silence existing genes or it can activate silent genes.

Among the concerns raised by the Petitioner is that the plants may be more susceptible to diseases or insects, may become weedier, or may produce more toxicants, anti-nutrients, or allergens.

Petitioners suggest that the original AgrEvo petition requesting nonregulated status for LLRICE06 and LLRICE62 ( [http://www.aphis.usda.gov/brs/aphisdocs/98\\_32901p.pdf](http://www.aphis.usda.gov/brs/aphisdocs/98_32901p.pdf)), “contained evidence of unintended effects with possible plant pest implications.”

- “One LibertyLink<sup>®</sup> rice variety had multiple rearranged insertions and different height properties than its parent.
- “Another LibertyLink<sup>®</sup> variety had less lectin and more phytic-acid than the parent variety.”
- “The parent M202 required no dry afterripening while 60% of LLRICE06 required one week or more of dry afterripening”
- Some LLRICE06 lines apparently differed from M202 in panicle and rice kernel characteristics
- No trypsin inhibitor was detected in either the parental or transformed lines

APHIS notes that the Petitioner has not noted any unintended changes that suggest plant pest effects. The so-called differences in height and panicle conformation were actually a result of less uniformity (greater standard deviation) than observed for the elite line from which they were derived, whereas the average of the measurements were not significantly different from the parent line. The seed afterripening values noted as a difference were well within the range of other rice lines. APHIS has evaluated the phenotype of the LibertyLink<sup>®</sup> rice plants and has concluded that the small differences between LibertyLink<sup>®</sup> rice and conventional lines are variations found within the range of other rice cultivars and not likely to pose a plant pest risk. APHIS notes that the Petitioner presents no data or information to support the assertion that the levels of phytic acid in LibertyLink<sup>®</sup> rice, warrant plant pest concerns. Petitioners speculate that lectin levels and trypsin inhibitor levels are abnormal and might result in susceptibility to insect damage but no such damage was observed in 3 years of field testing.

Moreover, subsequent to APHIS’s deregulation of LLRICE06 and LLRICE62, food and feed from these varieties were the subject of a completed consultation under FDA’s consultation procedures for foods derived from new plant varieties. Based on the information presented during the consultation, FDA did not question the crop developer’s determination that food and feed derived from LLRICE06 and LLRICE62 are not materially different in terms of composition or safety from food and feed derived from non-transgenic rice currently on the market (<http://www.cfsan.fda.gov/~rdb/bnfm063.html>).

APHIS acknowledges that insertion of DNA sequences can modify, interrupt, or silence existing genes or it can activate silent genes. However these effects also occur naturally and are exploited in conventional breeding programs. In traditional breeding the mutations may arise from errors in replication and recombination, insertion of naturally occurring genetic elements such as retrotransposons and transposons, gene silencing, and environmental mutagens such as radiation or chemical exposure (National Research Council, 2002). Petitioners have not convinced APHIS that there are unique risks posed

by unintended effects in LibertyLink<sup>®</sup> rice varieties or that there are any unintended effects that warrant plant pest concerns.

APHIS has thoroughly reviewed the petition and has determined that LibertyLink<sup>®</sup> rice is not a plant pest. Petitioners have not submitted any new information that convinces APHIS that LibertyLink<sup>®</sup> rice will increase the weediness of red rice or increase the evolution of herbicide resistant weeds above the baseline of current agricultural practices. As glufosinate is currently not used to control weeds on rice, in the worst case scenario should glufosinate no longer be useful to manage weeds in rice, management options would revert to the status quo. Commingling of genetically engineered crops with conventional and organic crops may decrease the value of the latter, but this impact is a market effect and not an environmental risk. EPA has thoroughly evaluated the impacts of glufosinate on endangered and non-target species and concluded that glufosinate can be used on rice as specified on the label with a certainty of no unreasonable harm. APHIS does not find that the arguments or information supplied to USDA by the Petitioner makes a compelling case that unintended effects of genetic engineering trigger plant pest concerns. No information supplied by the Petitioner has given APHIS a scientific basis to change the original decision on the regulatory status of LibertyLink<sup>®</sup> rice lines 06 and 62.

Therefore, APHIS affirmed its former determination that LLRICE06 and 62 are not plant pests and should not be subject to regulation under the Plant Protection Act.

Further, APHIS denies the petitioner's request to add LibertyLink<sup>®</sup> rice varieties to the list of organisms that are plant pests.

## **Additional References**

- Association of Official Seed Certifying Agencies. (2001). Genetic and Crop Standards. Moline, IL: 118 pp.
- Davis, L. C. (2006). "Genetic engineering, ecosystem change, and agriculture: an update." Biotechnology and Molecular Biology Review **1**(3): 87-102.
- Fernandez-Cornejo, J. and W. D. McBride (2002). Adoption of Bioengineered Crops, Economic Research Service/USDA. Agricultural Economic Report No. 810: 67 pp.
- Fischer, A. J. and A. Ramirez (1993). "Red rice (*Oryza sativa*): competition studies for management decisions." Int. J. Pest Management **39**: 133-138.
- Gianessi, L. P., C. S. Silvers, et al. (2002). Herbicide tolerant rice. Plant Biotechnology: Current and Potential Impact for improving Pest Management In U.S. Agriculture. An analysis of 40 case studies. Washington, D.C., National Center for Food and Agricultural Policy. 14pp
- Lopez-Martinez, N., J. G. Gonzalez-Gutierrez, et al. (2001). "Propanil activity, uptake and metabolism in resistant *Echinochloa* spp. biotypes." Weed Research **41**: 187-196.
- National Research Council. (2002). Environmental Effects of Transgenic Plants. Washington, D.C., National Academy Press. p.40
- U.S. EPA (2003). "Glufosinate Ammonium; Pesticide Tolerance Final Rule." Federal Register **68**(Sept. 29): 55833-55849.
- Wheeler, C., F.L.Baldwin, et al. (1997). Efficacy of Liberty (glufosinate) in Liberty-Tolerant Rice. Rice Research Studies 1997. Research Series 460. Fayetteville, Arkansas Agricultural Experiment Station.: 330-335.

USDA/APHIS Environmental Assessment

In response to Bayer CropScience Petition 06-234-01P seeking  
Extension of Determination of Non-regulated Status for  
Glufosinate Resistant rice, *Oryza sativa*, event LLRICE601

U.S. Department of Agriculture  
Animal and Plant Health Inspection Service  
Biotechnology Regulatory Services

# TABLE OF CONTENTS

<b>I.</b>	<b>SUMMARY .....</b>	<b>3</b>
<b>II.</b>	<b>INTRODUCTION.....</b>	<b>3</b>
	A. THE ANTECEDENT ORGANISMS.....	3
	B. PHOSPHINOTHRICIN N-ACETYLTRANSFERASE (PAT).....	4
	C. THE EXTENSION PROCESS.....	4
	D. COMPARISON OF LLRICE601 WITH DEREGULATED EVENTS -06 AND -62.....	4
	E. USDA-APHIS REGULATORY AUTHORITY .....	5
	F. ENVIRONMENTAL PROTECTION AGENCY (EPA) REGULATORY AUTHORITY .....	5
	G. FOOD AND DRUG ADMINISTRATION (FDA) REGULATORY AUTHORITY .....	6
<b>III.</b>	<b>PURPOSE AND NEED .....</b>	<b>6</b>
<b>IV.</b>	<b>ALTERNATIVES .....</b>	<b>6</b>
	A. NO ACTION ALTERNATIVE .....	6
	B. DENIAL OF PETITION: CONTINUATION AS A REGULATED ARTICLE.....	7
	C. DETERMINATION THAT LLRICE601 PLANTS ARE NO LONGER REGULATED ARTICLES .....	7
	D. PREFERRED ALTERNATIVE .....	7
<b>V.</b>	<b>AFFECTED ENVIRONMENT .....</b>	<b>7</b>
	A. CURRENT PRACTICES .....	7
	B. RICE BIOLOGY.....	8
	C. DETECTION OF LLRICE601 IN COMMERCIAL RICE.....	8
<b>VI.</b>	<b>POTENTIAL ENVIRONMENTAL IMPACTS.....</b>	<b>9</b>
	1. POTENTIAL IMPACTS FROM GENE INTROGRESSION FROM LLRICE601 INTO ITS SEXUALLY COMPATIBLE RELATIVES.....	9
	2. POTENTIAL IMPACTS BASED ON THE RELATIVE WEEDINESS OF LLRICE601 .....	10
	3. POTENTIAL IMPACT ON NON-TARGET ORGANISMS, INCLUDING BENEFICIAL ORGANISMS AND THREATENED OR ENDANGERED SPECIES .....	10
	4. POTENTIAL IMPACTS ON BIODIVERSITY .....	11
	5. POTENTIAL IMPACTS ON AGRICULTURAL PRACTICES.....	11
	6. POTENTIAL IMPACTS ON ORGANIC FARMING .....	12
	7. POTENTIAL IMPACTS ON RAW OR PROCESSED AGRICULTURAL COMMODITIES .....	12
<b>VII.</b>	<b>CONSIDERATION OF EXECUTIVE ORDERS, STANDARDS AND TREATIES RELATING TO ENVIRONMENTAL IMPACTS .....</b>	<b>13</b>
<b>VIII.</b>	<b>REFERENCES CITED .....</b>	<b>16</b>
<b>IX.</b>	<b>PREPARERS AND REVIEWERS.....</b>	<b>17</b>
<b>X.</b>	<b>AGENCY CONTACT .....</b>	<b>17</b>
<b>XI.</b>	<b>APPENDICES .....</b>	<b>18</b>
	APPENDIX I .....	18

## **I. Summary**

The Animal and Plant Health Inspection Service of the United States Department of Agriculture (USDA-APHIS), prepared an Environmental Assessment (EA) in response to a request (APHIS Number 06-234-01p ) from Bayer CropScience for an extension of a determination of non-regulated status issued for genetically engineered rice (*Oryza sativa* L.) lines LLRICE62 and LLRICE06 described in petition number 98-329-01p (AgrEvo 1998). Public comment was solicited on this assessment. LLRICE62 and LLRICE06 contain the *bar* gene from *Streptomyces hygroscopicus* HP632 which confers resistance to the herbicide glufosinate ammonium. The Bayer CropScience request claims that an additional rice line, LLRICE601, does not present a plant pest risk, and, therefore, LLRICE601 and its progeny derived from crosses with other non-regulated rice should no longer be regulated articles under regulations at 7 CFR Part 340, based on its similarity to the antecedent organisms.

LLRICE601 was field tested under nine notifications issued by APHIS (see Table 3, pg. 12, petition 06-234-01p). Field tests were conducted from 1998-2001. According to the developer, LLRICE601 was being developed as a backup line for LLRICE62, but commercial development was subsequently dropped. There was no indication from field data reports to suggest that LLRICE601 would behave any differently than lines LLRICE62 and LLRICE06 under field conditions. After review of the submitted petition, APHIS has concluded that LLRICE601 is similar to LLRICE62 and LLRICE06, and therefore has made the decision to extend a determination of nonregulated status to LLRICE601.

## **II. Introduction**

### **A. The Antecedent Organisms.**

LLRICE06 and LLRICE62 were developed to tolerate exposure to the herbicide glufosinate ammonium and thereby give rice growers another option for weed control during the growing season. Currently rice growers in the United States control weeds through a combination of herbicides, crop rotation, and cultural practices such as flooding and tillage.

Transformation events LLRICE06 and LLRICE62 were developed by introducing a single gene, the *bar* gene, into the varieties M202 and Bengal, respectively. The *bar* gene was derived from the soil-borne bacterium, *Streptomyces hygroscopicus* HP632. The *bar* gene encodes phosphinothricin-N-acetyltransferase (PAT), an enzyme which inactivates the herbicide glufosinate ammonium. Therefore the rice field could be treated with this herbicide to control weeds without damaging the rice crop.

In 1999, APHIS granted a determination of nonregulated status to LLRICE62 (Unique Identifier ACS-OS ØØ2-5) and LLRICE06 (Unique Identifier ACS-OS ØØ1-4). An EA was prepared (USDA-APHIS 1999) and during the 60-day public comment period in advance of the determination to deregulate these two lines, only four comments were

received, all in support of deregulation. Bayer also conducted a full consultation with the FDA on both events (FDA 1999a, 1999b)

Although these lines were deregulated in 1999 and EPA registered glufosinate for use on rice in 2002, neither line has ever been distributed for commercial use.

In addition to the LLRICE62 and LLRICE06 events, APHIS has evaluated petitions for nonregulated status for other crop species expressing the *bar* gene. Since 1995, APHIS deregulated 4 corn events, 5 rapeseed events, and 1 cotton event containing the *bar* gene. In addition, the agency has deregulated 6 corn events, 2 rapeseed events, 7 soybean events, and 1 sugarbeet event containing the very similar *pat* gene derived from *Streptomyces viridochromogenes* which also encodes the PAT protein. The majority of these glufosinate herbicide tolerant crop lines were originally developed by AgrEvo, whose parent company Hoechst AG merged with Rhone-Poulenc to form Aventis in 1999. Aventis was acquired by Bayer in 2002. *Bar* and *Pat* genes have been licensed for use by Syngenta, Dow, and Pioneer. Transgenic corn, canola, and cotton containing *bar* or *pat* genes have been commercialized beginning in 1996.

## **B. Phosphinothricin N-Acetyltransferase (PAT)**

This enzyme modifies glufosinate herbicides so that they are no longer toxic to plants (OECD 1999, OECD 2002). The gene is found in a wide range of microorganisms and encodes a well-characterized protein that has a history of safe use in agriculture. Reviews of 26 other events containing this protein have established the environmental safety of this protein (for more details see the preliminary risk assessment in Appendix 1).

## **C. The Extension Process.**

The extension process developed from APHIS' expectation that many regulated articles will be developed that differ insignificantly from others that have already been reviewed and granted nonregulated status. The aim of making comparisons between regulated articles and their antecedent organisms is to ensure that the new regulated articles in question raise no serious new issues meriting full review under the petition process. To qualify for the extension process (7 C.F.R. 340.6(e)), the regulated article must be similar to the previously deregulated, antecedent organism. Introduction of genetic material into the same species, or by a different transformation method, or that differs only in regulatory sequences, are examples of regulated articles that qualify for extension (see APHIS website, <http://www.aphis.usda.gov/brs/extback.html>). In the extension process, the developer submits a petition comparing the subject organism to the antecedent organism(s) while APHIS evaluates this comparison and determines whether any differences pose a significant plant pest risk.

## **D. Comparison of LLRICE601 with deregulated events -06 and -62.**

APHIS compared molecular and agronomic data from LLRICE601 to the previously deregulated rice events LLRICE06 and LLRICE62 as part of its preliminary risk assessment of the subject rice line (see Appendix 1). In summary, LLRICE601 is similar to LLRICE62 and 06. All contain the *bar* gene preceded by the 35S promoter, confer glufosinate tolerance, and exhibit no other significant phenotypic differences from the

corresponding parental comparator. There are a number of subtle differences listed in the preliminary risk assessment in Appendix 1, but none were deemed to be significant or to pose a plant pest risk.

### **E. USDA-APHIS Regulatory Authority**

APHIS regulations at 7 CFR Part 340, which were promulgated pursuant to authority granted by the Plant Protection Act (7 U.S.C. 7701-7772), regulate the introduction (importation, interstate movement, or release into the environment) of certain genetically engineered organisms and products. An organism is no longer subject to the regulatory requirements of 7 CFR Part 340 when it is demonstrated not to present a plant pest risk. A genetically engineered organism is considered a regulated article if the donor organism, recipient organism, vector, or vector agent used in engineering the organism belongs to one of the taxa listed in the regulation and is also a plant pest, or if there is reason to believe that it is a plant pest. In this submission, the plants have been genetically engineered using recombinant DNA techniques, and *Agrobacterium tumefaciens* is the donor of the *nos* DNA regulatory sequence that facilitates the expression of the introduced gene in the engineered plants. The *nos* sequence is from the soil-inhabiting bacterial plant pathogen, *Agrobacterium*, which is one of the listed taxa in the 7 CFR § 340.

Section 340.6(e)(2) of the regulations, entitled "Extensions to determinations of nonregulated status," provides that "a person may request that APHIS extend a determination of nonregulated status to other organisms. Such a request shall include information to establish the similarity of the antecedent organism and the regulated articles in question." If APHIS determines that the regulated article is sufficiently similar to an antecedent organism, the agency can grant the petition. In such a case, APHIS authorizations (i.e., permits or notifications) would no longer be required for field testing, importation, or interstate movement of the nonregulated article or its progeny.

This environmental assessment was prepared in accordance with: (1) The National Environmental Policy Act of 1969 (NEPA), as amended (42 U.C § 4321 *et seq.*); (2) regulations of the Council on Environmental Quality for implementing the procedural provisions of NEPA (40 CFR §§ 1500-1508); (3) USDA regulations and implementing NEPA (7 CFR § 1b); and (4) APHIS NEPA Implementing Procedures (7 CFR § 372).

### **F. Environmental Protection Agency (EPA) Regulatory Authority**

EPA has authority to regulate pesticides, including pesticidal substances produced by plants containing added genetic material (Plant-Incorporated Protectants) or PIPs, under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). Before a pesticide can be marketed and used in the United States, FIFRA requires that EPA evaluate the proposed pesticide to assure that its use will not pose unreasonable risks of harm to human health and the environment. This regulation involves an extensive review of health and safety information. Under the Federal Food, Drug, and Cosmetic Act (FFDCA) (21 U.S.C. 301 *et seq.*), pesticides added to (or contained in) raw agricultural

commodities generally are considered to be unsafe unless a tolerance or exemption from tolerance has been established. In the case of herbicide tolerant plants, EPA only regulates the chemical herbicide, not the herbicide tolerant plant. Glufosinate herbicide completed the EPA registration process on September 30, 2003 (68 FR 55833-55849). The EPA reviewed the submitted data, performed risk assessments, and approved the use of Liberty<sup>®</sup> on rice after careful consideration concluding that no "unreasonable harmful effects on endangered plants, other threatened or endangered species, or beneficial organisms" are expected from the use of Liberty<sup>®</sup>.

## **G. Food and Drug Administration (FDA) Regulatory Authority**

The FDA policy statement concerning regulation of products derived from new plant varieties, including those genetically engineered, was published in the Federal Register on May 29, 1992, and appears at 57 FR 22984-23005. Under this policy, FDA uses what is termed a consultation process to ensure that human food and animal feed safety issues or other regulatory issues (e.g., labeling) are resolved prior to commercial distribution of bioengineered food. For lines LLRICE06 and LLRICE62, Bayer completed a consultation with the FDA. LLRICE601, which was not intended for commercialization, was not submitted to FDA for evaluation under FDA's voluntary biotechnology consultation process. However, according to the FDA, Bayer has provided information to FDA about the safety of the PAT protein and molecular characterization of grain from LLRICE601. Based on the available data and information, FDA has concluded that the presence of this bioengineered rice variety in the food and feed supply poses no food or feed safety concerns (<http://www.cfsan.fda.gov/~lrd/biorice.html>).

## **III. PURPOSE and NEED**

APHIS prepared this EA before making a final determination on the status of LLRICE601 as a regulated article under APHIS regulations. In accordance with 7 CFR 340.6(e)(3) a preliminary decision based on a draft EA and preliminary risk assessment were published in the Federal Register on September 8, 2006, prior to the decision becoming final and effective. Additionally, the draft EA and Bayer petition requesting an extension of a determination of non-regulated status issued for genetically engineered rice (*Oryza sativa* L.) lines LLRICE62 and LLRICE06 were made publicly available on the APHIS website.

## **IV. ALTERNATIVES**

### **A. No Action Alternative**

Under the "no action" alternative, APHIS would take no action with respect to this petition at this time. LLRICE601 would continue to be a regulated article under the regulations at 7 CFR Part 340. Permits issued or notifications acknowledged by APHIS would still be required for introductions of LLRICE601 plants. APHIS might choose this alternative if it concluded that it could not, under all the circumstances, evaluate the petition adequately and reach a decision.

## **B. Denial of Petition: Continuation as a Regulated Article**

Under this alternative, APHIS would deny the petition. LLRICE601 would continue to be a regulated article under the regulations at 7 CFR Part 340. Permits issued or notifications acknowledged by APHIS would still be required for introductions of LLRICE601 plants. APHIS might choose this alternative if there were insufficient evidence to demonstrate the similarity of LLRICE601 to LLRICE62 or LLRICE06. If APHIS chooses this alternative, Bayer may subsequently submit a modified or separate petition for a determination of nonregulated status without prejudice. (7 C.F.R. 340.6 (e) (4)).

## **C. Determination that LLRICE601 plants are No Longer Regulated Articles**

Under this alternative, LLRICE601 would no longer be a regulated article under the regulations at 7 CFR Part 340. Permits issued or notifications acknowledged by APHIS would no longer be required for introductions of glufosinate tolerant rice derived from LLRICE601. APHIS might choose this alternative if there were sufficient evidence to demonstrate the similarity of LLRICE601 to LLRICE62 or LLRICE06 for which the agency had previously concluded presented no greater plant pest risk than rice developed in traditional breeding programs.

## **D. Preferred Alternative**

APHIS has chosen Alternative C as the preferred alternative. This is based upon the similarity of LLRICE601 to the antecedent organisms.

## **V. Affected Environment**

### **A. Current Practices**

In the United States, rice cultivation is concentrated in two regions:

- 1) the southern Mississippi River Valley, beginning in the Missouri “Bootheel” and moving south through Arkansas and Louisiana to the Gulf Coastal Plain into Texas
- 2) North Central California

In the South, long grain rice varieties are the principal types grown, whereas California grows primarily medium grain and short grain rice varieties. No GM varieties of rice are grown and distributed commercially. Herbicide tolerant rice lines resistant to imidazolinone herbicides have been developed by conventional mutation-induced breeding techniques. Marketed as Clearfield<sup>®</sup> varieties, the herbicide tolerant trait has been incorporated into long grain varieties for use in Southern states where red rice may be a weed pest. In 2006, Clearfield<sup>®</sup> varieties accounted for approximately 34% of the rice acreage grown in the South (Ouzts 2006). Crop rotation, irrigation management, and herbicides are used to control weeds in rice and no single weed management strategy is successful for control. A more detailed treatment of weed control can be found in Section

E of the Developer's original petition (AgrEvo 1998). Glufosinate is a low toxicity, non-selective herbicide. Glufosinate use on rice is regulated by the EPA. It has been registered for use on rice since 2002.

## **B. Rice Biology**

In this section of the environmental assessment, the biology of rice and plants related to rice are considered. Because the mechanism by which genes are moved from one flowering plant to another is through cross-pollination of sexually compatible plants, the plants with which rice can cross-pollinate are described. Below is an analysis of the biology of rice. This review focuses solely on rice in the United States. Other sources of information include a review prepared by the Organization for Economic Cooperation and Development (OECD), "Consensus Document on the Biology of *Oryza sativa* (Rice)" found at: [http://www.oecd.org/olis/1999doc.nsf/LinkTo/env-jm-mono\(99\)26](http://www.oecd.org/olis/1999doc.nsf/LinkTo/env-jm-mono(99)26) and the "Biology and Ecology of Rice (*Oryza sativa* L.) In Australia" found at <http://www.ogtr.gov.au/pdf/ir/biologyrice.pdf>.

Cultivated rice is included in the genus *Oryza* of the grass family (Poaceae). The genus *Oryza* contains twenty two species distributed through the tropical and subtropical regions of Asia, Africa, Central and South America, and Australia. Two species are cultivated and twenty are wild (Morishima, 1984; Vaughan et al., 2003). *O. sativa* is commonly referred to as Asian rice and is cultivated worldwide. The word "rice" generally indicates a plant and a crop of this species. *O. glaberrima* is commonly referred to as African rice and is cultivated in West and Central Africa. The genus *Oryza* is not native to the continental United States. One species, *Oryza latifolia* Desv. Broadleaf rice, is native to Puerto Rico. Only the single species, *Oryza sativa* is cultivated in the United States. Wild rice grown in the upper Midwest is another genus entirely and does not hybridize with *Oryza*.

Red rice is a weed complex associated with cultivated rice grown in the southern United States. It is a diverse complex of *Oryza sativa* ssp. *indica*, *O. sativa* ssp. *japonica*, *O. nivara* and *O. rufipogon* (Vaughan et al., 2001). Red rice has a red pericarp or seed coat, pubescent light-green leaves, pubescent seeds that are shed easily (shatter) and a dormancy mechanism that enables seed survival for extended periods under unfavorable soil and environmental conditions (Eastin, 1979; Diarra et al., 1985; Ladinsky, 1985). These characteristics are different from most cultivated rice which has a tan pericarp, does not shatter readily and has little if any seed dormancy. Red rice is also taller at maturity than most of the cultivars grown today. It can be a troublesome weed in rice growing operations in the southern United States.

## **C. Detection of LLRICE601 in commercial rice**

Bayer CropScience has learned that samples of commercial long grain rice were found to contain low levels of an event containing the *bar* gene. Molecular characterization has indicated that the detected event was LLRICE601 and not LLRICE62 or LLRICE06. Aventis discontinued field testing LLRICE601 in 2001.

## **VI. POTENTIAL ENVIRONMENTAL IMPACTS**

Potential impacts to be addressed in this EA are those that pertain to the use of LLRICE601 and its progeny in the absence of confinement.

### **1. Potential impacts from gene introgression from LLRICE601 into its sexually compatible relatives.**

In assessing the risk of gene introgression from LLRICE601 into its sexually compatible relatives, APHIS considered two primary issues: 1) the potential for gene flow and introgression; and 2) the potential impact of introgression.

Rice is not sexually compatible with plant species outside of the *Oryza* genus. In the United States, there are no sexually compatible species of *Oryza* other than *Oryza sativa*. Rice is primarily self-pollinating, and outcrossing rates usually occur at a very low rate (generally less than 1%) (OECD, 1999). The floral structure of *O. sativa* and the short viability of its pollen present biological barriers to cross-pollination (Gealy et al., 2003). A rice floret opens only once for a short period of time, usually for approximately an hour or less, during which time fertilization can occur. The stigma is fertilized by pollen produced by the same floret, therefore rice flowers are typically self-pollinated. Pollen viability is for no longer than five to ten minutes, but the stigma can remain viable for two to four days and can be fertilized by foreign pollen if for some reason it is not fertilized by its own pollen (Gealy et al., 2003). Gene introgression into commercial rice via pollen flow is therefore very unlikely. Due to the high self-pollinating characteristic of rice, the Association of Official Seed Certifying Agencies (AOSCA) certified seed regulations for foundation seed require a minimum isolation distance from other rice varieties of at least ten feet when ground drilled and 50 feet if ground broadcast (AOSCA, 2003).

In addition, another mechanism for gene escape is outcrossing to weedy/red rice. Species in the red rice complex, sometimes associated with the cultivation of rice, are the only species likely to hybridize with LLRICE601, but their competitiveness requires the same specific environmental conditions that are used for cultivation of commercial rice. Therefore, red rice is not considered weedy in other environments. Offspring from hybridization between LLRICE601 and red rice will not have enhanced competitive abilities except for glufosinate resistance. As other chemical and mechanical control practices are available besides glufosinate application, LLRICE601 is unlikely to increase the weediness potential of red rice.

Because red rice is the only species likely to hybridize with glufosinate tolerant rice, and control practices are available to mitigate any effects of introgression should it occur, there would be no impact related to outcrossing from deregulating this line (Alternative C). There would also be no impact from continuing to regulate the line (Alternatives A and B).

## **2. Potential impacts based on the relative weediness of LLRICE601**

Rice is a highly domesticated aquatic crop species, which grows exclusively in highly managed aquatic ecosystems. It is non-competitive with weed species and is self-pollinated. As a result, errant seed does not pose a threat to wild or managed, non-flooded ecosystems. Rice plants (*Oryza sativa*) growing unintentionally around rice growing areas are regarded as weeds (Vaughan and Morishima, 2003). Weedy rice can result from the escape of cultivated varieties into surrounding areas if conditions are suitable for establishment. It appears that weedy rice commonly evolves through the degeneration of domesticated rice (Vaughan et al., 2003). Weedy rice may be derived from hybridization between different cultivars, selection of weedy traits present in cultivars, relics of abandoned cultivars, or may have been brought into the growing region through contaminated seed stocks (Vaughan and Morishima, 2003). Weedy rice typically grows only as a component of agro-ecosystems where rice is grown or has been grown. It does not persist in environments inhospitable to rice cultivation.

Weedy red rice can be a major economic problem when it occurs in rice fields because it can lead to a loss in yield through competition with the desired cultivar as well as decreasing the value of the harvested grain. It is for this reason that many seed certification standards have a zero tolerance for red rice contamination in fields established for certified seed increases. For example see <http://www.moseed.org/rice.htm>.

No change in general agronomic traits (leaf color, shape, growth habit, days to pollen shed, days to maturity and seed germination rates) have been observed in LLRICE601 that might affect the plant's ability to persist in the environment (see Appendix 1). The presence of the *bar* gene in the rice seeds has not altered seed germination rates.

Because *O. sativa* does not persist in unmanaged ecosystems, it is only able to survive where rice is cultivated, and can be managed with existing cultural practices, there would be no weed impact from deregulating the genetically modified variety (Alternative C) and its subsequent release relative to the release of any conventional rice variety. There would also be no impact from continuing to regulate the line (Alternatives A and B).

## **3. Potential impact on non-target organisms, including beneficial organisms and threatened or endangered species**

The PAT protein is not known to have any toxic properties. The EPA, based on submitted toxicological data, established an exemption from the requirement of a tolerance for residues of PAT and the genetic material necessary for its production in all plants (USEPA 1997). Furthermore, LLRICE601 produces lower levels of PAT protein than the antecedent organism LLRICE62 which had no reported non-target effects. The level of protein in LLRICE601 seed has been estimated to be 120 ng/g fw which is only 0.000034% of the crude rice protein.

Analysis of both qualitative and quantitative information from the petition and published data supports the developer's conclusion that the unconfined release of LLRICE601 and its progeny would not harm any non-target or Federally listed (or proposed) threatened or

endangered species. An analysis of Threatened and Endangered Species was conducted for the six major rice producing States (Arkansas, California, Louisiana, Mississippi, Missouri and Texas) and for three minor production States (Florida, Oklahoma, Tennessee) using the U.S. Fish and Wildlife database <http://ecos.fws.gov/ecos/index.do> and NatureServe database: <http://www.natureserve.org/explorer/>. The analysis found that there are a few plant species that are sometimes associated with rice fields in California. In California and the Gulf Coast States there are a number of Threatened and Endangered Animal species that could visit or inhabit rice fields from time to time. Most of these are various bird species that could feed in and around rice production areas. In California the Giant Garter Snake can live in rice fields and Vernal Pool Fairy Shrimp are known to inhabit rice fields. In Texas the Attwater's Greater Prairie Chicken is known to inhabit fallow rice fields. The engineered rice would not be expected to affect any of these species or other species that visit or inhabit rice fields since the PAT protein has been shown to be neither toxic nor allergenic (Hérouet, et al., 2005). Likewise, based on the phenotypic similarity to conventional rice varieties in all respects other than glufosinate tolerance, APHIS concluded that LLRICE601 would not have any more impact on habitat of listed (or proposed) threatened or endangered species than conventional rice.

BRS has reviewed the data in accordance with a process mutually agreed upon with the U.S. Fish and Wildlife Service to determine when a consultation is needed as required under Section 7 of the Endangered Species Act. APHIS reached a determination that the release of LLRICE601 would have no effect on listed (or proposed) species and consequently a written concurrence or formal consultation with Fish and Wildlife Service is not required for this EA.

Under any of the alternatives, there would be no impact on nontarget organisms or Federally-listed (or proposed) threatened or endangered species.

#### **4. Potential impacts on biodiversity**

Analysis of available information indicates that LLRICE601 exhibits no traits that would cause increased weediness and that its unconfined cultivation should not lead to increased weediness of other cultivated rice or other sexually compatible relatives. LLRICE601 line exhibited no change in disease susceptibility, and it is unlikely to harm non-target organisms common to the agricultural ecosystem or threatened or endangered species recognized by the U.S. Fish and Wildlife Service. Based on this analysis, there is no apparent potential for significant impact to biodiversity under Alternative C. If APHIS chooses alternative A or B, there would also be no impact on biodiversity.

#### **5. Potential impacts on agricultural practices**

If LLRICE601 were to be grown commercially, the effect from introducing LLRICE601 into the environment on agricultural practices would be no different than for the deregulated lines. As the trait expressed is resistance to glufosinate and the level of resistance is similar to that of the other two lines, there should be no difference in impact

on standard agricultural practices in rice cultivation and controlling volunteer rice. See Appendix I for more details.

## **6. Potential impacts on organic farming**

The National Organic Program (NOP) is administered by USDA's Agricultural Marketing Service (AMS). Organic production operations must develop and maintain an organic production system plan approved by their accredited certifying agent in order to obtain certification. Organic certification of a production or handling operation is a process claim, not a product claim. Organic certification involves oversight by an accredited certifying agent of the materials and practices used to produce or handle an organic agricultural product. Oversight by a certifying agent includes an annual review of the certified operation's organic system plan and on-site inspections of the certified operation and its records.

The organic system plan enables the production operation to achieve and document compliance with the National Organic Standards, including the prohibition on the use of excluded methods. Excluded methods include a variety of methods used to genetically modify organisms or influence their growth and development by means that are not possible under natural conditions or processes. Although the National Organic Standards prohibit the use of excluded methods, they do not require testing of inputs or products for the presence of excluded methods, unless a certifying agent has reasonable suspicion that a prohibited substance or excluded method was used. The presence of a detectable residue of a product of excluded methods alone does not necessarily constitute a violation of the National Organic Standards. Planting of genetically-modified seed would render the organic crop non-organic and require it to be sold as conventional. The status of the organic operation depends on the operator's foreknowledge of the origin and status of the seed planted. The duty of an organic grower to develop and maintain an organic production system that meets the relevant regulatory standards will not change regardless of which alternative is selected. Therefore, granting the extension of nonregulated status (Alternative C) will have no significant impact on organic farming. Likewise there will be no significant impact from alternatives A and B.

## **7. Potential impacts on raw or processed agricultural commodities**

APHIS analysis of data on agronomic performance, disease and insect susceptibility, and compositional profiles of LLRICE601 and its parent variety indicate no significant differences between the two that would be expected to cause either a direct or indirect plant pest effect on any raw or processed plant commodity from deregulation of LLRICE601. Similarly, there were no significant differences between LLRICE62 and LLRICE06 and their corresponding parent varieties. There would be no impacts on raw or processed agricultural commodities from deregulating the genetically modified variety (Alternative C). There would also be no impact from regulating the line (Alternatives A and B).

## **VII. CONSIDERATION OF EXECUTIVE ORDERS, STANDARDS AND TREATIES RELATING TO ENVIRONMENTAL IMPACTS**

Executive Order (EO) 12898, "Federal Actions To Address Environmental Justice in Minority Populations and Low-Income Populations," requires Federal agencies to conduct their programs, policies and activities that substantially affect human health or the environment in a manner so as not to exclude persons and populations from participation in or benefiting from such programs. It also enforces existing statutes to prevent minority and low-income communities from being subjected to disproportionately high and significant human health or environmental effects. Each alternative was analyzed in its ability to affect minority and low-income populations. None of the alternatives was found to pose disproportionately high or significant human health or environmental effects to any specific minority or low-income group.

EO 13045, "Protection of Children from Environmental Health Risks and Safety Risks," acknowledges that children may suffer disproportionately from environmental health and safety risks because of their developmental stage, greater metabolic activity levels and behavior patterns, as compared to adults. The EO (to the extent permitted by law and consistent with the agency's mission) requires each Federal agency to identify, assess and address environmental health risks and safety risks that may disproportionately affect children. None of the alternatives are expected to have disproportionately high or significant human health or environmental effects on children.

EO 13112, "Invasive Species," states that federal agencies take action to prevent the introduction of invasive species and provide for their control and to minimize the economic, ecological and human health impacts that invasive species cause. Rice is not invasive and is widely prevalent in the U.S. Based on the data submitted by the applicant and reviewed by APHIS, the engineered plant is not different in any fitness characteristics from its parent that might increase its invasive potential.

Executive Order 12114, "Environmental Effects Abroad of Major Federal Actions" requires Federal officials to take into consideration any potential environmental effects outside the U.S., its territories and possessions that result from actions being taken. APHIS has given this due consideration and does not expect a significant environmental impact outside the United States should an extension of non-regulated status be granted for LLRICE601 or if one of the other alternatives is chosen. It should be noted that all the considerable, existing national and international regulatory authorities and phytosanitary regimes that currently apply to introductions of new rice cultivars internationally, apply equally to those covered by an APHIS determination of non-regulated status under 7 CFR Part 340. Any international traffic of LLRICE601 subsequent to an extension of non-regulated status for LLRICE601 would be fully subject to national phytosanitary requirements and be in accordance with phytosanitary standards developed under the International Plant Protection Convention (IPPC).

The purpose of the IPPC "is to secure a common and effective action to prevent the spread and introduction of pests of plants and plant products, and to promote appropriate

measures for their control” (<https://www.ippc.int/IPP/En/default.jsp>). The protection it affords extends to natural flora and plant products and includes both direct and indirect damage by pests, including weeds. The IPPC has set a standard for the reciprocal acceptance of phytosanitary certification among the nations that have signed or acceded to the Convention (137 countries as of April 2005). In April, 2004, a standard for pest risk analysis (PRA) of living modified organisms (LMOs) was adopted at a meeting of the governing body of the IPPC as a supplement to an existing standard, International Standard for Phytosanitary Measure No. 11 (ISPM-11; Pest Risk Analysis for Quarantine Pests). The standard acknowledges that all LMOs will not present a pest risk, and that a determination needs to be made early in the PRA for importation as to whether the LMO poses a potential pest risk resulting from the genetic modification. APHIS pest risk assessment procedures for bioengineered organisms are consistent with the guidance developed under the IPPC. In addition, issues that may relate to commercialization and transboundary movement of particular agricultural commodities produced through biotechnology are being addressed in other international forums and through national regulations.

The Cartagena Protocol on Biosafety is a treaty under the United Nations Convention on Biological Diversity (CBD) that established a framework for the safe transboundary movement, with respect to the environment and biodiversity, of LMOs, which includes those modified through biotechnology. The Protocol came into force on September 11, 2003 and 134 countries are Parties to it as of July 13, 2006 (see <http://www.biodiv.org/biosafety>). Although the United States is not a party to the CBD, and thus not a party to the Cartagena Protocol on Biosafety, U.S. exporters will still need to comply with domestic regulations that importing countries that are Parties to the Protocol have put in place to comply with their obligations. The first intentional transboundary movement of LMOs intended for environmental release (field trials or commercial planting) will require consent from the importing country under an advanced informed agreement (AIA) provision, which includes a requirement for a risk assessment consistent with Annex III of the Protocol, and the required documentation. LMOs imported for food, feed or processing (FFP) are exempt from the AIA procedure, and are covered under Article 11 and Annex II of the Protocol. Under Article 11, Parties must post decisions to the Biosafety Clearinghouse database on domestic use of LMOs for FFP that may be subject to transboundary movement. To facilitate compliance with obligations to this protocol, the U.S. Government has developed a website that provides the status of all regulatory reviews completed for different uses of bioengineered products (<http://usbiotechreg.nbii.gov>). This data will be available to the Biosafety Clearinghouse. APHIS continues to work toward harmonization of biosafety and biotechnology consensus documents, guidelines and regulations, including within the North American Plant Protection Organization (NAPPO), which includes Mexico, Canada, and the United States and in the Organization for Economic Cooperation and Development. NAPPO has completed three modules of a standard for the *Importation and Release into the Environment of Transgenic Plants in NAPPO Member Countries* (see <http://www.nappo.org/Standards/Std-e.html>). APHIS also participates in the North American Biotechnology Initiative (NABI), a forum for information exchange and cooperation on agricultural biotechnology issues for the U.S., Mexico and Canada. In

addition, bilateral discussions on biotechnology regulatory issues are held regularly with other countries including: Argentina, Brazil, Japan, China, and Korea. Many countries, e.g. Argentina, Australia, Canada, China, Japan, Korea, Philippines, South Africa, Switzerland, the United Kingdom.

## VIII. REFERENCES CITED

- AgrEvo. USA** (1998) Company Petition 98-329-01p Determination of Nonregulated Status for Rice Genetically Engineered for Glufosinate Herbicide Tolerance. [http://www.aphis.usda.gov/brs/aphisdocs/98\\_32901p.pdf](http://www.aphis.usda.gov/brs/aphisdocs/98_32901p.pdf)
- AOSCA** (2003) "Yellow Books" 2003 Operational Procedures, Crop Standards and Service Programs. AOSCA, Meridian, Idaho
- Diarra A, Smith RJ, Talbert RE** (1985) Growth and morphological characteristics of red rice (*Oryza sativa*) biotypes. *Weed Science* **33**: 310-314
- Eastin EF** (1979) Selected bibliography of red rice and other wild rices (*Oryza* spp.). *In* Miscellaneous-Publication,-Texas-Agricultural-Experiment-Station. Texas-Agricultural-Experiment-Station, pp 60 pp
- FDA** (1999a) Agency Response Letter: Consultation Completed <http://www.cfsan.fda.gov/~rdb/bnfl063.html>
- FDA** (1999b) Agency Summary Memo: <http://vm.cfsan.fda.gov/~rdb/bnfm063.html>.
- FDA** (2006) U.S. Food and Drug Administration's Statement on Report of Bioengineered Rice in the Food Supply (<http://www.cfsan.fda.gov/~lrd/biorice.html>)
- Gealy DR, Mitten DH, Rutger JN** (2003) Gene flow between red rice (*Oryza sativa*) and herbicide-resistant rice (*O. sativa*): implications for weed management. *Weed Technology* **17**: 627-645
- Herouet,-C; Esdaile,-D-J; Mallyon,-B-A; Debruyne,-E; Schulz,-A; Currier,-T; Hendrickx,-K; Klis,-R-J-van-der; Rouan,-D.** 2005. Safety evaluation of the phosphinothricin acetyltransferase proteins encoded by the pat and bar sequences that confer tolerance to glufosinate-ammonium herbicide in transgenic plants. *Regulatory-Toxicology-and-Pharmacology*. 2005; 41(2): 134-149
- Ladinsky G** (1985) Founder effect in crop-plant evolution. *Economic Botany* **39**: 191-199
- Morishima H** (1984) Wild plants and domestication, Chapter 1. *In* S Tsunoda, N Takahashi, eds, *Biology of Rice*, Vol 7. Elsevier, Amsterdam, pp 3-30
- OECD** (1999) Environmental Health and Safety Publications. Series on Harmonization of Regulatory Oversight in Biotechnology. No. 11. Consensus Document on General Information Concerning the Genes and their Enzymes that Confer Tolerance to Phosphinothricin Herbicide. ([http://www.olis.oecd.org/olis/1999doc.nsf/LinkTo/env-jm-mono\(99\)13](http://www.olis.oecd.org/olis/1999doc.nsf/LinkTo/env-jm-mono(99)13))
- OECD** (1999) Consensus Document on the biology of *Oryza sativa* (Rice). *In* Series on Harmonization of Regulatory Oversight in Biotechnology, pp 52
- Ouzts, R** (2006). Horizon Ag New Horizons Newsletter. Vol 1. No 1.p.3 <http://www.orygen.net/newsletter/august2006.pdf#search=%22clearfield%20rice%20acres%20planted%22>
- USDA-APHIS.** 1999. Availability of Determination of Nonregulated Status for Rice Genetically Engineered for Glufosinate Herbicide Tolerance. *Federal Register*: April 27, Volume 64, No. 80, pp 22595
- USDA-APHIS.** 1999. Environmental Assessment forPetition 98-329-01p. Determination of Nonregulated Status for Rice Genetically Engineered for Glufosinate Herbicide Tolerance. [http://www.aphis.usda.gov/brs/aphisdocs2/98\\_32901p\\_com.pdf](http://www.aphis.usda.gov/brs/aphisdocs2/98_32901p_com.pdf)

- USEPA.** 1997. Phosphinothricin acetyltransferase and the genetic material necessary for its production in all plants-exemption from the requirement of a tolerance on all raw agricultural commodities. Federal Register: April 11, 1997, Volume 62, No. 70, pp. 17717-17720.
- Vaughan, L.K., B.V. Ottis, C.C. Bormans, A.M. Prazak-Havey, C.H. Sneller, J.M. Chandler, and W.D. Park.** 2001. Is all red rice found in commercial rice really *Oryza sativa*? *Weed Sci.* 49:468-476
- Vaughan DA, Morishima H** (2003) Biosystematics of the genus *Oryza*. Chapter 1.2. *In* CW Smith, RH Dilday, eds, *Rice, Origin, History, Technology, and Production*. John Wiley and Sons Inc., Hoboken, NJ, pp 27-65
- Vaughan DA, Morishima H, Kadowaki K** (2003) Diversity in the *Oryza* genus. *Current Opinion in Plant Biology* 6: 139-146

## **IX. PREPARERS AND REVIEWERS**

### **Biotechnology Regulatory Services**

Cindy Smith, Deputy Administrator

Rebecca Bech, Associate Deputy Administrator for Emerging and International Programs

### **BRS, Environmental Risk Analysis Division**

Neil Hoffman, Ph.D., Director (Preparer of EA)

Michael Watson, Ph.D. Supervisory Biotechnologist (Reviewer)

Levis Handley, Ph.D., Senior Biotechnologist (Reviewer)

Rebecca Stankiewicz-Gabel, Ph.D., Senior Biotechnologist (Reviewer)

### **BRS, Policy and Coordination Division**

John Turner, Ph.D., Director

Michael Wach, Ph.D., Supervisory Regulatory Analyst (Reviewer)

## **X. AGENCY CONTACT**

Steven M Bennett, Acting Document Control Officer

USDA, APHIS, BRS

4700 River Road, Unit 147

Riverdale, MD 20737-1237

Phone: (301) 734-5672

Fax: (301) 734-8669

steven.m.bennett@aphis.usda.gov

## **XI. APPENDICES**

### **Appendix I.**

#### **Summary of the evidence that the inadvertent release of LLRICE601 poses no environmental concerns**

##### **Background**

In 1999, APHIS granted a determination of nonregulated status to two herbicide tolerant rice transformation events designated LLRICE62 (Unique Identifier ACS-OSØØ2-5) and LLRICE06 (Unique Identifier ACS-OSØØ1-4). These lines were produced by inserting the *bar* gene from *Streptomyces hygroscopicus* HP632 which encodes the enzyme phosphinothricin N-acetyltransferase (PAT). PAT provides resistance to the herbicide glufosinate by metabolizing the active ingredient of the herbicide to an inactive form. During the 60-day public comment period in advance of the determination to deregulate these two lines, only four comments were received, all in support of deregulation. Although these lines were deregulated in 1999 and EPA registered glufosinate for use on rice in 2002, neither line has ever been distributed for commercial use.

In addition to the LLRICE62 and LLRICE06 events, APHIS has evaluated petitions for nonregulated status for other crop species expressing the *bar* gene. Since 1995, APHIS deregulated 4 corn events, 5 rapeseed events, and 1 cotton event, containing the *bar* gene. In addition, the agency has de-regulated 6 corn events, 2 rapeseed events, 7 soybean events, and 1 sugarbeet event containing the very similar *pat* gene derived from *Streptomyces viridochromogenes* which also encodes the PAT protein. The majority of these glufosinate herbicide tolerant crop lines were originally developed by AgrEvo, whose parent company Hoechst AG merged with Rhone-Poulenc to form Aventis in 1999. Aventis was acquired by Bayer in 2002.

##### **Phosphinothricin N-Acetyltransferase (PAT)**

This enzyme modifies glufosinate herbicides so that they are no longer toxic to plants (OECD 2002). The gene is found in a wide range of microorganisms and encodes a well characterized protein that has a history of safe use in agriculture. Reviews of 26 other events containing this protein have established the environmental safety of this protein. PAT protein lacks sequence homology to known toxins and is unlikely to be an allergen because it is rapidly digested in simulated gastric and intestinal fluids, is unstable to heat greater than 40°C, and lacks glycosylation sites. Numerous feeding studies have been conducted in mice, birds, and rabbits which document that the protein is neither toxic to humans or animals (OECD 1999). As such the EPA, based on submitted toxicological data, established an exemption from the requirement of a tolerance for residues of the phosphinothricin acetyltransferase (PAT) and the genetic material necessary for its production in all plants. Thus, the potential for toxicity or allergenicity to humans or nontarget organisms for PAT protein expressed in rice lines containing event LLRICE601 is considered remote.

### **Comparison of LLRICE601 with deregulated events -06 and -62.**

Rice lines LLRICE601, -06, and -62 were field tested under the appropriate APHIS authorizations over several growing seasons in the United States prior to the decision of the developer to seek nonregulated status for lines LLRICE06 and LLRICE62. LLRICE601 was maintained by the developer as a backup line. Field evaluations of LLRICE06 and 62 are described in documentation submitted by the developer in support of the petition to APHIS for a determination of nonregulated status ([http://www.aphis.usda.gov/brs/aphisdocs/98\\_32901p.pdf](http://www.aphis.usda.gov/brs/aphisdocs/98_32901p.pdf)). For these two lines it was specifically concluded that they posed no greater plant pest risk than rice developed in traditional breeding programs and were therefore granted nonregulated status by APHIS. APHIS further notes that since the date of the determination of nonregulated status for lines LLRICE06 and -62 in 1999, other regulatory authorities (Canada, Russia, and Argentina) have approved its use in the environment and/or as food and feed. Based on a comparison of the data submitted in the petition for granting nonregulated status to LLRICE06 and 62 and data submitted by Bayer on August 4, 2006, regarding the molecular characterization of event LLRICE601, APHIS has identified the following similarities and differences between the rice lines:

### **Similarities between rice lines LLRICE601 and -06; -62**

1. Rice line LLRICE601 is similar to lines LLRICE06 and LLRICE62. All contain a single transgene, *bar* driven by the 35S CaMV (Cauliflower Mosaic Virus) promoter.
2. In no cases were antibiotic resistance markers (kanamycin for -06 and -62; spectinomycin for 601) from the respective plasmid vectors integrated into the genome.
3. Molecular and genetic characterization of all three lines indicate that the *bar* gene is stably inherited.
4. All are resistant to the herbicide glufosinate.
5. All differ from their traditional counterpart by only the addition of the *bar* gene sequence into the genome, and the expression of the PAT protein.
6. -601 and -62 produce a single anti-PAT immunoreactive peptide of the same apparent molecular weight when analyzed on Western blots.

### **Differences between rice lines LLRICE601, -06, and -62.**

1. The DNA construct was introduced into the LLRICE06 and -62 by direct gene transfer but was introduced into -601 by *Agrobacterium*-mediated transformation. Both direct gene transfer and *Agrobacterium*-mediated transformation are standard practices for introduction of genetic material into plant genomes and therefore APHIS does not consider this difference significant. Both are well characterized transformation methods which integrates the donor genes into the chromosome of the recipient plant cell. The donor DNA sequences are stably and irreversibly integrated into the plant's chromosomal or organelle DNA, where they are maintained and inherited as any other genes of the plant cell.

2. The 35S CaMV promoter is slightly longer for -601 versus -06 and -62. APHIS does not consider this difference significant. The promoter in -601 has been used in other events where no unusual effects were observed and which have completed USDA and FDA regulatory review. The 35S CaMV promoter is among the most common gene sequences used in genetically engineered plants and has a history of safe use.
3. LLRICE601 uses the *nos* (nopaline synthase) terminator while -06 and -62 use the 35S CaMV terminator. APHIS does not consider this difference significant. The *nos* terminator does not encode a protein or functional RNA, is widely used in genetic engineering, and has been approved in a number of deregulated products, for example LLCotton25 and MON810 corn.
4. LLRICE06, -62, and -601 represent different varieties of rice which have each been transformed with the *bar* gene. LLRICE06 was transformed into the medium grain variety M202, LLRICE62 was transformed into the medium grain variety Bengal, and LLRICE601 was transformed into the long grain variety Cocodrie. APHIS does not consider this difference significant. During the evaluation of a petition for nonregulated status, APHIS considers the fact that a particular event may be crossed into other genetic backgrounds and grants nonregulated status to the subject of the petition and all progeny bred from the deregulated lines. Indeed LLRICE62 has been introgressed into other long grain varieties through conventional breeding and these all have nonregulated status.
5. There are minor differences in the level of PAT protein expressed between all three events (the two nonregulated events LLRICE06 and -62 and the regulated event -601). In the seed, the level of PAT protein in LLRICE601 is below the level in -06 and -62. In the leaf tissues, the level of PAT protein in -601 is much less than the level in -62 but is slightly higher than the level in -06. APHIS does not consider these differences significant. As the PAT protein levels of -601 are below those of -62 and no unintended effects were observed on non-target organisms in -62, no unintended effects on non-target organisms due to differences in PAT expression levels are expected in -601.
6. The sequence of the PAT protein produced in LLRICE601 is identical to the sequence produced in the approved cotton line LLCotton25. These sequences vary from the LLRICE06 and -62 PAT proteins by a single amino acid at position 2 where the former have an aspartic acid residue and the latter have a serine. APHIS does not consider this difference to be significant because lines corresponding to both versions of the protein have completed USDA and FDA regulatory review.

### **Molecular Characterization of LLRICE601**

Details on molecular characterization of LLRICE601 were submitted to APHIS in a report, Molecular Characterization of Glufosinate-Tolerant rice transformation event LLRICE601, dated August 4, 2006. Bayer included a Southern blot analysis of DNA from lines containing the event LLRICE601 consistent with the conclusion that only a single *bar* gene flanked by an intact 35S promoter and a truncated *nos* terminator were inserted into the genome. In addition, the Southern blot revealed extra bands for the 35S

CaMV promoter suggesting a second copy of the promoter inserted elsewhere in the genome. The random insertion of an extra 35S promoter fragment in the rice genome is unlikely to have any consequence as the effectiveness of the promoter is dependent on it inserting close enough to DNA encoding a functional gene. At a low frequency, it could potentially insert near enough to another gene to alter the expression of a native rice gene. Alternatively, it could integrate within a native rice gene and disrupt its function. As submitted phenotypic data (see Whole Plant Evaluation below) revealed no apparent differences between LLRICE601 and the parent variety, if there are any changes in gene expression, those changes do not appear to pose a plant pest risk. Southern blots were provided that were consistent with the conclusion that no coding sequences from the vector, including the spectinomycin gene, were integrated into the rice genome.

To characterize the insertion site of the P35S-*bar*-nos gene, flanking DNA was sequenced and analyzed for the presence of transcriptional and translational regulatory elements (CART, TATA-boxes, ribosome binding sites, and polyadenylation signals). The absence of most or all of these regulatory elements in each putative open reading frame in the flanking regions is strong evidence that the expression of newly created proteins derived from the 5-prime or 3-prime junction region is highly unlikely. Sequence comparison of flanking DNA to genomic DNA conclusively identified the insertion site on chromosome 12. At the insertion site, no homology was found to any known gene, cDNA, or expressed sequence tag (EST) indicating that the insertion did not disrupt a native rice gene.

Stability of the inserted *bar* gene cassette was evaluated over multiple generations. The results obtained demonstrate the stability of the event LLRICE601 at the genomic level.

### **Whole plant evaluations of LLRICE601.**

Details on field evaluations of lines LLRICE601-5001, -5201, -5401, and 5601 were submitted to APHIS in a report, Agronomic Performance of glufosinate-tolerant rice transformation event LLRICE601, dated August 4, 2006. At the T3 generation, these four inbred lines of LLRICE601 and the parent variety, Cocodrie, were tested for two seasons in multi-location replicated trials (AR, LA, MS, and TX). The last testing was completed in 2001. LLRICE601 exhibited consistent commercial level resistance to glufosinate whereas Cocodrie was sensitive. To measure response to rice pathogens sheath blight, panicle blight, and rotten neck blast, LLRICE601 was tested alongside Cypress, the standard for US long grain rice disease screening, and was found to have similar responses. For attributes related to weediness potential (seed germination, dormancy, and panicle shattering) LLRICE 601 and Cocodrie were virtually indistinguishable. Likewise, for all other agronomic properties measured including, panicle morphology, days to maturity, lodging tendency, yield, and grain characteristics (a total of 86 attributes were measured) LLRICE601 was comparable to Cocodrie. Thus, when grown in the field, LLRICE601 was nearly indistinguishable from Cocodrie. In field tests in 2000, LLRICE601 appeared to be shorter than Cocodrie. However the two lines were not significantly different in height during the 2001 growing season. Similarly, LLRICE62 and LLRICE06 were not significantly different from their respective parental comparators in any agronomic characters except for glufosinate tolerance. All the

characteristics measured for LLRICE601, LLRICE06, and LLRICE62 were in the expected range of conventional rice except for the glufosinate resistance trait.

### **Compositional analysis of LLRICE601**

Aventis looked at grain crude fat/oil, protein, ash, fiber, carbohydrates, minerals, vitamins, amino acids, and fatty acids. No significant differences were observed between transgenic rice, transgenic rice sprayed with herbicide, and non-transgenic rice for any of the parameters measured.

### **No evidence for inadvertent effects in LLRICE601.**

Morphological and biochemical data reveal no significant differences between LLRICE601 and Cocodrie, indicating that the insertion of the CaMV35S promoter-*bar* gene- *nos* terminator and the additional 35S promoter have created no apparent unintended effects in LLRICE601.

### **Environmental Impacts from the introduction of LLRICE601**

LLRICE601 grows normally and appears to interact with other organisms in the environment in ways that should not present plant pest risks or significant impacts on the environment. Given that the PAT protein is non-toxic, no increased risk to nontarget organisms or threatened and endangered species is anticipated by the introduction of LLRICE601. Rice is not listed as a common, serious, or principal weed or a weed of current or potential importance in the United States and Canada. Species in the red rice complex sometimes associated with the cultivation of rice, are the only species likely to hybridize with LLRICE601, but their competitiveness requires the same specific environmental conditions that are used for cultivation of commercial rice. Therefore, red rice is not considered weedy in other environments. Offspring from hybridization between LLRICE601 and red rice will not have enhanced competitive abilities except for glufosinate resistance. As other chemical and mechanical control practices are available besides glufosinate application, LLRICE601 is unlikely to increase the weediness potential of red rice.

### **Effects on Agricultural Practices from the introduction of LLRICE601**

Because this event is similar to the two approved lines (LLRICE62 and LLRICE06) the effect from introducing LLRICE601 into the environment on agricultural practices would be no different than for the deregulated lines. As the trait expressed is resistance to glufosinate and the level of resistance is similar to that of the other two lines, there should be no difference in impact on standard agricultural practices in rice cultivation and controlling volunteer rice. Specifically, if volunteer rice were to appear in agricultural fields it can be managed in the same way that any glufosinate-tolerant volunteers are currently managed. For example in rotation practices where a soybean crop might follow a rice crop, volunteer rice is usually treated with post-emergent soybean herbicides for controlling grasses. Herbicides such as quizalofop [Assure II], fluazifop [Fusilade] or sethoxydim [Poast] are commonly employed. Volunteer rice can also be controlled with preplant burndown applications of paraquat [Gramoxone Extra] and glyphosate [Roundup Ultra or Roundup WeatherMax]. LLRICE601 is also sensitive to the herbicides used in the Clearfield<sup>®</sup> system, including imazethapyr [Newpath] and

imazamox [Beyond]. In the case where Roundup Ready® soybeans or cotton would follow a rice crop, glyphosate could be used.

In addition, because it was concluded in the review of LLRICE 62 or LLRICE06 that this trait does not contribute to increased weediness, and based on agronomic data supplied by the developer for LLRICE601, it can also be concluded that this identical trait does not contribute to increased weediness in line LLRICE601.

## **Conclusion**

LLRICE601 has undergone extensive field testing and characterization.

1. It exhibits no plant pathogenic properties and is no more susceptible to disease than the reference rice variety, Cypress.
2. It is no more likely to become a weed than other herbicide tolerant rice varieties developed by traditional plant breeding. Rice is not a weed pest in the U.S. and there is no reason to believe that resistance to glufosinate herbicides would enable rice to become a weed pest.
3. While it is possible that the *bar* gene could be transmitted to red rice by pollen gene flow, control practices are available to mitigate any effects of introgression should it occur.
4. LLRICE601 is not toxic and exhibits no potential to harm organisms beneficial to the agricultural system. Likewise, it is not expected to harm threatened or endangered species.
5. LLRICE601 is not different than conventional rice with respect to a wide variety of morphological, agronomic, and biochemical attributes and therefore is no more likely to cause damage to raw or processed agricultural commodities than rice varieties developed by conventional breeding.
6. LLRICE601 has a high degree of similarity to two antecedent organisms (LLRICE06 and LLRICE62) which have been granted nonregulated status. All characteristics measured for LLRICE06, LLRICE62, and LLRICE601 were in the expected range of conventional rice with the exception of the glufosinate resistance trait.

APHIS believes that the available evidence stated above supports the conclusion that LLRICE601 is likely to be as safe as LLRICE06, -62, and conventionally bred rice lines. Therefore APHIS concludes that the inadvertent release of LLRICE601 poses no environmental concerns.

## **Selected resource materials:**

USDA-APHIS documents:

- Petition submitted by the developer AgrEvo to APHIS in 1998 for LLRICE06 and LLRICE62. [http://www.aphis.usda.gov/brs/aphisdocs/98\\_32901p.pdf](http://www.aphis.usda.gov/brs/aphisdocs/98_32901p.pdf)
- APHIS decision documents at the time of the determination of nonregulated status in 1999. [http://www.aphis.usda.gov/brs/aphisdocs2/98\\_32901p\\_com.pdf](http://www.aphis.usda.gov/brs/aphisdocs2/98_32901p_com.pdf)

EPA:

- USEPA. 1997. Phosphinothricin acetyltransferase and the genetic material necessary for its production in all plants-exemption from the requirement of a tolerance on all raw agricultural commodities. Federal Register: April 11, 1997, Volume 62, No. 70, pp. 17717-17720.

Food and Drug Administration documents for LLRICE06 and 62:

- Status: Consultation Completed
- Response Letter: <http://www.cfsan.fda.gov/~rdb/bnfl063.html>
- Summary Memo: <http://vm.cfsan.fda.gov/~rdb/bnfm063.html>

OECD 1999. Environmental Health and Safety Publications. Series on Harmonization of Regulatory Oversight in Biotechnology. No. 11. Consensus Document on General Information Concerning the Genes and their Enzymes that Confer Tolerance to Phosphinothricin Herbicide. ([http://www.olis.oecd.org/olis/1999doc.nsf/LinkTo/env-jm-mono\(99\)13](http://www.olis.oecd.org/olis/1999doc.nsf/LinkTo/env-jm-mono(99)13))

OECD 2002. Environmental Health and Safety Publications. Series on Harmonization of Regulatory Oversight in Biotechnology. No. 25. Module II. Phosphinothricin. ([http://www.olis.oecd.org/olis/2002doc.nsf/43bb6130e5e86e5fc12569fa005d004c/c351fd9d795e54c1c1256bae0051a2a8/\\$FILE/JT00125605.PDF](http://www.olis.oecd.org/olis/2002doc.nsf/43bb6130e5e86e5fc12569fa005d004c/c351fd9d795e54c1c1256bae0051a2a8/$FILE/JT00125605.PDF))

## **Determination of an extension of nonregulated status of rice line LLRICE601 from LLRICE62 and LLRICE06**

In response to the petition 06-234-01p from Bayer CropScience, APHIS has determined that rice line LLRICE601 and progeny derived from it are no longer regulated articles under 7 CFR 340 *et seq.* This determination is based on APHIS' analysis of field, greenhouse and laboratory data, references provided in the petition, and other relevant information as described in this environmental assessment that indicate that LLRICE601 is similar to the antecedent organisms LLRICE62 and LLRICE06 which were granted nonregulated status in response to APHIS petition number 98-329-01p.

Like the antecedent organisms LLRICE62 and LLRICE06, rice line LLRICE601 has been genetically engineered to contain the *bar* gene isolated from the bacterium *Streptomyces hygroscopicus*, under the control of a 35S promoter sequence derived from cauliflower mosaic virus (35S CaMV). The *bar* gene encodes a phosphinothricin acetyltransferase (PAT) enzyme that confers tolerance to the herbicide glufosinate. LLRICE601 and LLRICE62 produce a single PAT protein of the same apparent molecular weight, as demonstrated by Western blotting. LLRICE06 does not produce sufficient protein for the size to be determined by this method. The level of expression of the PAT protein produced in LLRICE601 plants falls between that of the two antecedent organisms LLRICE62 and LLRICE06.

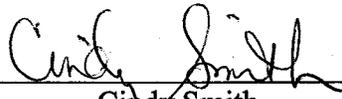
The DNA construct was introduced into LLRICE06 and LLRICE62 by direct gene transfer, but was introduced into LLRICE601 by *Agrobacterium* mediated transformation. Both direct gene transfer and *Agrobacterium* mediated transformation are standard practices for introduction of genetic material into plant genomes; APHIS does not consider this difference significant.

The 35S CaMV promoter is among the most common gene sequences used in genetically engineered plants and has a long history of safe use. The 35S CaMV promoter is slightly longer for LLRICE601 than it is for LLRICE06 or LLRICE62. APHIS does not consider this difference significant. The promoter in LLRICE601 has been used in other events that have APHIS and FDA approval, and no unusual effects have been observed in those events. LLRICE601 uses the *nos* (nopaline synthase) terminator, while LLRICE06 and LLRICE62 use the 35S CaMV terminator. The function of the terminator is to provide a polyadenylation site, a necessary part of the mRNA transcript of the gene. In LLRICE601, the *nos* terminator is truncated. However, the PAT protein is still made, so the truncation does not affect the function of the transgene. The *nos* terminator is widely used in genetic engineering, and has been approved in a number of deregulated products, *e.g.*, LLCotton25, MON810 corn, BT11 corn, Round-up Ready® Soybean 40-3-2, and many insect resistant potato lines. APHIS does not consider LLRICE601's use of a different terminator than the antecedent organisms to be a significant difference because both sequences provide the same function.

LLRICE06 was originally genetically engineered into the medium grain variety M202, and LLRICE62 was originally genetically engineered into the medium grain variety Bengal and has since been bred into other rice varieties, including long grain varieties. LLRICE601 was originally genetically engineered into the long grain variety Cocodrie. APHIS does not consider this difference significant.

The sequence of the PAT protein produced in LLRICE601 is identical to the sequence produced in the approved cotton line LLCotton25. These sequences vary from the PAT proteins in LLRICE06 and LLRICE62 by a single amino acid at position 2, where the former have an aspartic acid residue and the latter have a serine. APHIS does not consider this difference to be significant because lines corresponding to both versions of the protein have undergone applicable reviews by APHIS and FDA.

Accordingly, we have concluded that rice line LLRICE601 is similar to the antecedent organisms in APHIS petition number 98-329-01p, and we have reached a determination that rice line LLRICE601 should no longer be regulated under 7 CFR part 340. Permits or acknowledged notifications that were previously required for environmental release, importation, or interstate movement under those regulations no longer will be required for LLRICE601 and its progeny. Importation of seeds and other propagative material of LLRICE601 would still be subject to APHIS foreign quarantine notices at 7 CFR Part 319 and the Federal Seed Act regulations at 7 CFR Part 201.



Cindy Smith

Deputy Administrator  
Biotechnology Regulatory Services  
Animal and Plant Health Inspection Service  
U.S. Department of Agriculture

Date: NOV 24 2006