Notices

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This section of the FEDERAL REGISTER contains documents other than rules or proposed rules that are applicable to the public. Notices of hearings and investigations, committee meetings, agency decisions and rulings, delegations of authority, filing of petitions and applications and agency statements of organization and functions are examples of documents appearing in this section.

DEPARTMENT OF AGRICULTURE

Animal and Plant Health Inspection Service

[Docket No. 97-094-2]

Monsanto Co.; Availability of Determination of Nonregulated Status for Potato Genetically Engineered for Insect and Virus Resistance

AGENCY: Animal and Plant Health Inspection Service, USDA.

ACTION: Notice.

SUMMARY: We are advising the public of our determination that certain potato lines developed by the Monsanto Company, which have been genetically engineered for insect and virus resistance, are no longer considered regulated articles under our regulations governing the introduction of certain genetically engineered organisms. Our determination is based on our evaluation of data submitted by the Monsanto Company in its petition for a determination of nonregulated status, an analysis of other scientific data, and our review of comments received from the public in response to a previous notice announcing our receipt of the Monsanto Company's petition. This notice also announces the availability of our written determination document and its associated environmental assessment and finding of no significant impact.

EFFECTIVE DATE: December 3, 1998.

ADDRESSES: The determination, an environmental assessment and finding of no significant impact, the petition, and all written comments received regarding the petition may be inspected at USDA, room 1141, South Building, 14th Street and Independence Avenue SW., Washington, DC, between 8 a.m. and 4:30 p.m., Monday through Friday, except holidays. Persons wishing to inspect those documents are requested to call before visiting on (202) 690-2817 to facilitate entry into the reading room.

FOR FURTHER INFORMATION CONTACT: Dr. James White, Biotechnology and Biological Analysis, PPQ, APHIS, 4700 River Road Unit 147, Riverdale, MD 20737-1236; (301) 734-5940. To obtain a copy of the determination or the environmental assessment and finding of no significant impact, contact Ms. Kay Peterson at (301) 734-4885; e-mail: Kay.Peterson@.usda.gov.

SUPPLEMENTARY INFORMATION:

Background

On July 23, 1997, the Animal and Plant Health Inspection Service (APHIS) received a petition (APHIS Petition No. 97-204-01p) from the Monsanto Company (Monsanto) of St. Louis, MO, seeking a determination that seven NewLeaf® Plus Russet Burbank potato lines, which have been genetically engineered for resistance to the Colorado potato beetle (CPB) and potato leaf roll virus (PLRV), do not present a plant pest risk and, therefore, are not regulated articles under APHIS' regulations in 7 CFR part 340. Subsequently, Monsanto requested that all but two (RBMT21-129 and RBMT21-350) of the NewLeaf® Plus Russet Burbank potato lines be withdrawn from consideration in the subject petition.

On November 20, 1997, APHIS published a notice in the Federal Register (62 FR 61961-61962, Docket No. 97-094-1) announcing that the Monsanto petition had been received and was available for public review. The notice also discussed the role of APHIS, the Environmental Protection Agency, and the Food and Drug Administration in regulating the subject potato lines and food products derived from them. In the notice, APHIS solicited written comments from the public as to whether these potato lines posed a plant pest risk. The comments were to have been received by APHIS on or before January 20, 1998. During the designated 60-day comment period, APHIS received 14 comments from the following: Potato farmers and processors; State and national trade associations; a State congressional delegation; the U.S. Department of Agriculture's Agricultural Research Service; a research entomologist from a university agricultural research center; and a European crop research institute. All of the comments were in support of the subject petition.

Analysis

Russet Burbank potato lines RBMT21-129 and RBMT21-350 have been genetically engineered to contain the *cryIIIA* gene derived from *Bacillus thuringiensis* subsp. *tenebrionis* (*Btt*), which encodes an insecticidal protein that is effective against CPB, and the *orf1/orf2* gene derived from PLRV, which imparts resistance to PLRV. In addition to the *cryIIIA* gene and the *orf1/orf2* gene, these potato lines contain the *nptII* gene, which encodes the NPTII protein used as a selectable marker in the initial stages of plant transformation. The subject potato lines were developed through use of the *Agrobacterium tumefaciens* transformation method, and expression of the added genes is controlled in part by gene sequences derived from the plant pathogens *A. tumefaciens* and figwort mosaic virus.

The subject potato lines have been considered regulated articles under APHIS' regulations in 7 CFR part 340 because they contain gene sequences derived from plant pathogens. However, evaluation of field data reports from field tests of these potato lines conducted under APHIS permits and notifications since 1994 indicates that there were no deleterious effects on plants, nontarget organisms, or the environment as a result of the environmental release of the subject potato plants.

Determination

Based on its analysis of the data submitted by Monsanto, a review of other scientific data and field tests of the subject potato lines, and an analysis of comments from the public on the subject petition, APHIS has determined that Russet Burbank potato lines RBMT21-129 and RBMT21-350:

(1) Exhibit no plant pathogenic properties; (2) are no more likely to become a weed than pest-resistant potato lines developed by traditional breeding techniques; (3) are unlikely to increase the weediness potential for any other cultivated or wild species with which they can interbreed; (4) will not cause damage to raw or processed agricultural commodities; and (5) will not harm threatened or endangered species or other organisms, such as bees, that are beneficial to agriculture, or have an adverse impact on the ability to control nontarget insect pests.

Therefore, APHIS has concluded that the subject Russet Burbank potato lines and any progeny derived from crosses with other potato varieties will be as safe to grow as potatoes that are not subject to regulation under 7 CFR part 340.

The effect of this determination is that Monsanto's Russet Burbank potato lines RBMT21–129 and RBMT21–350 are no longer considered regulated articles under APHIS' regulations in 7 CFR part 340. Therefore, the requirements pertaining to regulated articles under those regulations no longer apply to the field testing, importation, or interstate movement of the subject potato lines or their progeny. However, importation of the subject potato lines or seeds capable of propagation are still subject to the restrictions found in APHIS' foreign quarantine notices in 7 CFR part 319.

National Environmental Policy Act

An environmental assessment (EA) has been prepared to examine the potential environmental impacts associated with this determination. The EA was prepared in accordance with: (1) The National Environmental Policy Act of 1969, as amended (NEPA) (42 U.S.C. 4321 *et seq.*), (2) regulations of the Council on Environmental Quality for implementing the procedural provisions of NEPA (40 CFR parts 1500–1508), (3) USDA regulations implementing NEPA (7 CFR part 1b), and (4) APHIS' NEPA Implementing Procedures (7 CFR part 372). Based on that EA, APHIS has reached a finding of no significant impact (FONSI) with regard to its determination that Monsanto's Russet Burbank potato lines RBMT21–129 and RBMT21–350 and lines developed from them are no longer regulated articles under its regulations in 7 CFR part 340. Copies of the EA and the FONSI are available upon request from the individual listed under **FOR FURTHER INFORMATION CONTACT.**

Done in Washington, DC, this 14th day of December 1998.

Joan M. Arnoldi,

Acting Administrator, Animal and Plant Health Inspection Service.

[FR Doc. 98–33435 Filed 12–16–98; 8:45 am]

BILLING CODE 3410–34–P

USDA/APHIS Petition 97-204-01p for Determination of Nonregulated Status for Colorado Potato Beetle-and Potato Leaf Roll Virus-Resistant Potato Lines RBMT21-129 and RBMT21-350

Environmental Assessment and
Finding of No Significant Impact

December 1998

The Animal and Plant Health Inspection Service (APHIS) of the U. S. Department of Agriculture has prepared an environmental assessment prior to issuing a determination of nonregulated status for genetically engineered Colorado potato beetle- and potato leaf roll resistant potato transformation events designated: RBMT21-129 and RBMT21-350. APHIS received a petition from the Monsanto Company regarding the status of these lines as regulated articles under APHIS regulations at 7 CFR Part 340. APHIS has conducted an extensive review of the petition and supporting documentation, as well as other relevant scientific information. Based upon the analysis documented in this environmental assessment, APHIS has reached a finding of no significant impact on the environment from its determination that these two Colorado potato beetle- and potato leaf roll- resistant transformation events shall no longer be regulated articles.

Rebecca A. Bech
Scientific Services
Plant Protection and Quarantine
Animal and Plant Health Inspection Service
U.S. Department of Agriculture
Date: DEC 3 1998

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I. SUMMARY

The Animal and Plant Health Inspection Service (APHIS), United States Department of Agriculture (USDA), has prepared an Environmental Assessment (EA) prior to making a determination on the regulated status of two genetically engineered Colorado potato beetle (CPB) and potato leaf roll virus (PLRV) resistant transgenic potatoes. The Monsanto Company (hereafter referred to as Monsanto), the developer of these CPB- and PLRV-resistant potatoes, petitioned APHIS requesting a determination on the regulated status of these transgenic potatoes. They have been regulated articles under APHIS regulations. Monsanto has petitioned APHIS for a determination that these potatoes do not present a plant pest risk, and should therefore no longer be regulated articles under APHIS regulations 7 CFR Part 340.

The CPB- and PLRV-resistant potatoes have been developed as an alternative means of providing season-long control of the two damaging pests of potato crops, Colorado potato beetle, *Leptinotarsa decemlineata* (Say), and potato leaf roll virus. The resistance genes conferring resistance to CPB and PLRV were introduced via genetic engineering techniques. These techniques enabled the developer to express in potato plants the gene *cryIIIA* from the bacterium *Bacillus thuringiensis* subsp. *tenebrionis* encoding a highly selective insecticidal protein; the open reading frame *(orf)* 1 and 2 gene from PLRV that encode a protease and replicase; and the selectable marker gene encoding the enzyme neomycin phosphotransferase (nptII). All the genes were introduced via a well-characterized procedure that results in direct introduction of genes into plant genomes using a nonphytopathogenic strain of the bacterium *Agrobacterium tumefaciens*.

This EA specifically addresses the potential for impacts to the human environment through the use in agriculture of these two potato lines. It does not address the separate issue of the potential use of the plant pesticide CryIIIA or the *orf* 1 and 2 genes in conjunction with these lines. The United States Environmental Protection Agency (EPA) has authority over the use in the environment of all pesticidal substances under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). The Food and Drug Administration (FDA) has authority over food and feed issues of all genetically improved plants used as food or feed.

The EAs that were prepared before granting the initial permits for field trials and subsequent trials of these transgenic potatoes address questions pertinent to plant pest risk issues concerning the conduct of field trials under physical and reproductive confinement. However, they do not address several issues that are of relevance to the unconfined cultivation of these transgenic potatoes. With respect to these new issues, APHIS concludes the following:

1. CPB- and PLRV-resistant potatoes exhibit no plant pathogenic properties. Although pathogenic organisms were used in their development, these potato plants are not infected nor can they incite disease in other plants.

2. CPB- and PLRV-resistant potatoes are no more likely to become weeds than similar pest-resistant potatoes developed by traditional breeding techniques. Potatoes are not a weed pest in the U.S., and there is no reason to believe that resistance to CPB and PLRV would enable potatoes to become a weed.

3. Multiple reproductive barriers ensure that gene introgression from these CPB- and PLRV-resistant potatoes into wild or cultivated sexually-compatible plants are unlikely. Even in the unlikely event of gene introgression, this should not increase the weediness potential of resulting progeny or have an adverse impact on biodiversity than similar pest-resistant potatoes developed by traditional breeding techniques.

4. Except for being pest resistant, these potatoes are substantially equivalent to nontransgenic tubers and, therefore, APHIS can foresee no adverse impacts on raw or processed agricultural commodities.

5. CPB- and PLRV-resistant potatoes exhibit no significant potential to either harm organisms beneficial to the agricultural ecosystem, to have an adverse impact on the ability to control nontarget insect pests, or to harm threatened and endangered species.

Therefore, after a review of the available evidence, APHIS believes that these CPB- and PLRV-resistant potatoes will be just as safe as nontransgenic potatoes that are typically grown using other methods to control the CPB or PLRV, and which are not subject to regulation under 7 CFR Part 340. APHIS concludes that there should be no significant impact on the human environment if CPB- and PLRV-resistant potatoes were no longer considered regulated articles under regulations at 7 CFR Part 340.

II. BACKGROUND

A. Development of CPB- and PLRV-resistant Potatoes.

These transgenic potatoes were developed to provide genetic resistance to two of the most severe potato pests, CPB and PLRV These pests are often control by the application of chemical insecticides. In the case of PLRV, the insecticides are targeted to kill the aphid vector of the virus. The gene, *cryIIIA*, conferring CPB resistance, originally isolated from the soil bacterium *Bacillus thuringiensis* subsp. *tenebrionis* (*Btt*), encodes a crystalline protein (delta-endotoxin) designated CryIIIA protein. This protein exhibits highly selective insecticidal activity against a narrow range of coleopteran insects, particularly CPB. Upon ingestion of this protein by susceptible insects, feeding is inhibited with disruption of the midgut epithelium, which eventually results in death. The coding region of the *cryIIIA* gene was modified with plant-preferred amino acid codons for optimal expression in plants. The CryIIIA protein's synthesis is directed by the ribulose biphosphate carboxylase small subunit promoter that directs the production of the delta-endotoxin to the leaves.

The gene conferring resistance to PLRV are the *orf* (open reading frames) 1 and 2 from PLRV. This gene encodes the helicase and replicase domains required for viral RNA synthesis. Production of the *orf* 1 and 2 gene is directed by the figwort mosaic caulimovirus (FMV) promoter that directs the production of this gene to most all plant tissues.

CPB- and PLRV-resistant potatoes have also been transformed with a selectable marker that enables identification and selection of the transformed plant cells during tissue culture. The neomycin phosphotransferase (nptII), isolated from a common human colon bacterium, *Escherichia coli*, encodes an enzyme that confers resistance to antibiotics kanamycin and neomycin and is used in the selection of transformed cells.

The genes were introduced into CPB- and PLRV-resistant potatoes via an *Agrobacterium*-mediated transformation protocol. This is a well-characterized procedure that has been widely used for over a decade for introducing various genes of interest directly into plant genomes.

APHIS authorized the first field testing these two lines starting in late 1993 and they have been field tested in the major potato growing regions of the United States under the following APHIS authorization numbers (93-362-01r, 94-217-02r, 94-342-01r, 97-017-03r, 98-068-08n, 98-068-09n, 98-068-10n, 98-076-05n, 98-085-24n, 98-085-25n, 98-092-02n, 98-092-03n, 98-121-08n, 98-132-09n, 98-141-03n, and 98-141-04n). The subject lines of CPB- and PLRV-resistant potatoes have been evaluated extensively to confirm that they exhibit the desired agronomic characteristics and do not present a plant pest risk. Although the field tests have been conducted in agricultural settings, the conditions for the tests have stipulated physical and reproductive confinement from other plants.

B. APHIS Regulatory Authority.

APHIS regulations at 7 CFR Part 340, which were promulgated pursuant to authority granted by the Federal Plant Pest Act, as amended (7 U.S.C. 150aa-150jj) and the Plant Quarantine Act, as amended (7 U.S.C. 151-164a, 166-167) 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. CPB- and PLRV-resistant potatoes described in the Monsanto petition have been considered regulated articles because they contain the *orf* 1 and 2 gene from PLRV, noncoding DNA regulatory sequences derived from plant pathogens, and the vector agent used to deliver the plasmid vector is a plant pathogen.

Section 340.6 of the regulations, entitled "Petition Process for Determination of Nonregulated Status", provides that a person may petition the Agency to evaluate submitted data and determine that a particular regulated article does not present a plant pest risk, and therefore should no longer be regulated. If APHIS determines that the regulated article is unlikely to present a greater plant

pest risk than the unmodified organism, the Agency can grant the petition in whole or in part. As such, APHIS permits would no longer be required for field testing, importation, or interstate movement of the non-regulated article or its progeny.

C. EPA and FDA Regulatory Authority

These genetically engineered potato lines are also currently subject to regulation by other agencies. The EPA is responsible for the regulation of pesticides under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), as amended (7 U.S.C. 136 *et seq.*). FIFRA requires that all pesticides, including insecticides, be registered prior to distribution or sale, unless exempt by EPA regulation. Under the Federal Food, Drug, and Cosmetic Act (FFDCA), as amended (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. Residue tolerances for pesticides are established by EPA under the FFDCA, and the FDA enforces the tolerances set by the EPA.

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. Safety concerns for human and animal consumption of products with kanamycin resistance are also specifically addressed by the FDA in 21 CFR Parts 173 and 573.

III. PURPOSE AND NEED

APHIS has prepared this EA before making a determination on the status of CPB- and PLRV-resistant potatoes as regulated articles under APHIS regulations. The developer of CPB- and PLRV-resistant potatoes, Monsanto, submitted a petition to USDA, APHIS requesting that APHIS make a determination that these CPB- and PLRV-resistant potatoes shall no longer be considered regulated articles under 7 CFR Part 340.

This EA was prepared in compliance with the National Environmental Policy Act (NEPA) of 1969 as amended, (42 USC 4321 *et seq.*) and the pursuant implementing regulations (40 CFR 1500-1508; 7 CFR Part 1b; 7 CFR Part 372).

IV. ALTERNATIVES

A. No Action.

Under the Federal "no action" alternative, APHIS would not come to a determination that CPB- and PLRV-resistant potatoes are not regulated articles under the regulations at 7 CFR Part 340. APHIS permits or notifications would still be required for introductions of CPB- and PLRV-resistant potatoes. APHIS might choose this alternative if there was insufficient evidence

to demonstrate the lack of plant pest risk from uncontained cultivation of these CPB- and PLRV-resistant potatoes.

B. Determination that CPB- and PLRV-resistant Potatoes Are No Longer Regulated Articles.

Under this alternative, these CPB- and PLRV-resistant potatoes would no longer be regulated articles under the regulations at 7 CFR Part 340 and as such APHIS permits or notifications would no longer be required for introductions of these potatoes. A basis for this determination would include a "Finding of No Significant Impact" under the National Environmental Policy Act of 1969, as amended (42 USC 4321 *et seq.*; 40 CFR 1500-1508; 7 CFR Part 1b; 7 CFR Part 342).

V. AFFECTED ENVIRONMENT AND POTENTIAL ENVIRONMENTAL IMPACTS

This EA addresses potential environmental impact from a determination that these CPB- and PLRV- resistant potatoes should no longer be considered regulated articles under APHIS regulations at 7 CFR Part 340. This EA discusses the genetic modification, and the potential environmental impacts that might be associated with the unconfined cultivation of CPB- and PLRV-resistant potatoes.

Additional technical information is included in the determination document appended to this EA and is incorporated by reference. This includes detailed discussions of the biology of potato, the genetic components used in the construction of CPB- and PLRV-resistant potatoes, and the analyses that lead APHIS to conclude that CPB- and PLRV-resistant potatoes have no potential to pose plant pest risks and are as safe to use as insect- and virus-resistant potatoes developed by traditional breeding.

A. Potential for CPB- and PLRV-resistant Potatoes to Exhibit Increased Weediness Relative to Traditional Potatoes.

APHIS evaluated whether the CPB- and PLRV-resistant potatoes are any more likely than nontransgenic potatoes to present a plant pest risk as weeds. Most definitions of weediness stress the undesirable nature of weeds from the point of view of humans, from this starting point, individual definitions differ in approach and emphasis (Baker, 1965; de Wet and Harlan, 1975; Muenscher, 1980). Baker (1965) described the ideal characteristics of weeds, and although these characteristics have been criticized, no more broadly accepted set of characteristics have been defined by ecologists (Williamson, 1994). In our view, there is no formulation that is clearly superior at this time. Keeler (1989) and Tiedje et al. (1989) have adapted and analyzed Baker's list to develop admittedly imperfect guides to the weediness potential of transgenic plants; both authors emphasize the importance of looking at the parent plant and the nature of the specific genetic changes. Cultivated potato lacks most of these "weedy" characteristics (Keeler, 1989). Potato is not listed as a common, serious or principal weed or a weed of current or potential

importance in the United States or Canada (Holm et al., 1991; Muenscher, 1980; USDA, 1971; Weed Science Society of America, 1992).

It is unlikely that expression of the *cryIIIA* and *orf* 1 and 2 gene in the CPB- and PLRV- resistant potatoes will provide a competitive advantage sufficient to cause these to become more "weedy" than nontransformed potatoes. These CPB- and PLRV-resistant potato plants were routinely compared to nontransgenic potatoes during field trials for differences in physical characteristics, disease susceptibility, and insect susceptibility. The field data reports indicated no obvious differences in the number of volunteers, emergence from seed potatoes, and disease and insect susceptibility (other than to target pests). In addition, nontransgenic plants treated with insecticides to control CPB and the aphid vector of PLRV are no more "weedy" or difficult to control than any other potatoes. In addition, traditional resistance genes to these two pests have been identified and used in certain potato cultivars. The USDA Germplasm Resources Information Network (GRIN, 1994) contains accessions of at least 15 different species in the genus *Solanum* L., subgenus *Potato*, section *Petota* reputed to have resistance to CPB and collected in countries (i.e., Costa Rica, Guatemala, Mexico, and the United States) where CPB is listed as a pest. The Shepody potato cultivar is resistant to PLRV induced net necrosis (Jayasinghe and Salazar, 1998), somaclonal resistance to field infection by PLRV has been identified (Kawchuk *et al.* 1997), and other resistant cultivars or accessions have been identified (Ross, 1986).

Based on evaluation of the available literature and data submitted by Monsanto, APHIS concludes that the CPB- and PLRV-resistant potatoes are no more likely than nontransgenic potatoes containing traditional resistance genes to CPB or PLRV to present a plant pest risk as a weed.

B. Potential Environmental Impact Associated with Potential Gene Introgression from CPB- and PLRV-resistant Potatoes to Sexually Compatible Plants

APHIS evaluated the potential for gene flow from CPB- and PLRV-resistant potatoes to other cultivated and wild relatives and the potential impacts that this might have on weediness potential of progeny.

1) Potential for gene introgression into other potato cultivars.

All cultivated potatoes in the U.S. belong to the species *Solanum tuberosum*. Since the parental cultivar that was transformed (Russet Burbank) is male sterile as are the transgenic plants, it is unlikely that pollen from the transgenic will pollinate any potatoes that would result in viable offspring. Any transgenic seedlings would be unlikely to persist in the environment because of cultivation or herbicide usage in rotation crops during normal production practices. Introgression into another cultivar would be unlikely to have an impact on cultivated potatoes in the U.S., because these are vegetatively propagated mostly from certified seed potatoes that are grown under conditions to ensure genetic purity.

2) Potential for gene transfer to wild or free-living sexually compatible species occurring in the United States.

In the unlikely event that male-fertile progeny were produced from CPB- and PLRV-resistant potatoes as a result of introgression into another potato cultivar, APHIS evaluated the potential for gene transfer to wild or free-living sexually-compatible species occurring in the United States, and the environmental impacts associated with such events. Tuber-bearing *Solanum* species, including *S. tuberosum*, are unsuccessful in forming natural hybrids with the native or introduced weeds of *Solanum* species in the U.S. that do not bear tubers. Successful gene introgression into tuber-bearing *Solanum* species occurring in the United States (i.e., *S. jamesii*, *S. fendleri*, and *S. pinnatisectum*) is also virtually excluded due to constraints of geographical isolation and other biological barriers to natural hybridization.

3) Potential for gene introgression into wild relatives outside of the United States and associated potential impacts.

This determination does not carry with it any foreign safety presumption, since our authority only extends to the borders of the United States and its territories and possessions. It is unlikely that cultivation of these two transformation events will impact any sexually-compatible *Solanum sp.* because these potatoes are male sterile.

CPB- and PLRV-resistant potatoes are likely only to be cultivated where CPB and PLRV are serious pests and in environments suitable to these potatoes. Hanneman (1994) thoroughly evaluated the potential for gene exchange between cultivated *S. tuberosum* and wild and cultivated relatives in the Central American center of diversity. He concluded that there is little threat of introduction of genes into the two tuber-bearing wild *Solanum* species occurring in Costa Rica because of differences in their habitats and probable differences in endosperm balance number (EBN). Mexico has the greatest number of wild species known in North or Central America, and many species native to Mexico also exist in Guatemala. Introgression into many of these species is also inhibited by incompatible EBNs (OECD, 1997). The possibility exists for introgression into wild species with an EBN equal to that of cultivated potato (4EBN) and into local *S. tuberosum* ssp. *andigena* cultivars that are cultivated in Costa Rica, Mexico and Guatemala. These species are not listed as serious, principal or common weeds in Mexico by Holm et al. (1991), even though a few of the wild species are described as weeds by Hanneman (1994). But because they are generally found or cultivated at higher elevations than commercial *S. tuberosum*, significant introgression into these wild species and local cultivars is unlikely.

C. Development of Viral Symptoms on Some Transgenic Plants

APHIS noted that a small percentage of transgenic plants developed symptoms when intentionally inoculated with PLRV. These data showed that depending on the year, location of the test, and the transformation line, upwards of 18% of the plants showed developed leaf roll symptoms on their leaves. In 1996 under field conditions of natural infections, 60% of the control Russet

Burbank developed symptoms, while only 1.4% line RBMT21-129 plants showed symptoms. The other two lines showed no symptoms under these conditions.

APHIS then asked Monsanto to address whether the development of symptoms in these plants was a result of the challenge virus overcoming the resistance or the loss of the resistance gene. Monsanto submitted data that supported their contention that a resistance breaking strain had not developed and that the failure the *orf* 1 and 2 transgene to protect the plant is probably due to high inoculum pressure or weak expression of the *orf* 1 and 2 gene. Monsanto also demonstrated that in the symptom-containing transgenic plants, the transgenes had not be lost.

D. Potential for the Appearance of New Plant Viruses

As mentioned above, these transgenic potatoes were developed by engineering the *orf* 1 and 2 gene from PLRV into Russet Burbank cultivar. As part of its analysis, APHIS evaluated whether the expression of this viral gene might present some unusual circumstances that could lead to the appearance of new plant viruses.

In the course of the infection of a plant cell by more than a single type of virus, it is possible for some of the constituents of the viruses to become mismatched. Such occurrences can lead to recombination of the nucleic acid genome. It is theoretically possible for new plant viruses to arise in these transgenic potato transformation events through the recombination and APHIS considered this issue carefully in making its determination. A technical discussion of this issue is found in the Determination document appended to this EA. After careful consideration of the physical and biological properties of PLRV, the other viruses that infect potatoes, and the properties of the protease/replicase gene, APHIS concluded that it is unlikely that new viruses will appear as result of recombination as a consequence of the widespread cultivation these potato transformation events. (Transcapsidation is not an issue since it only involves viral coat protein). APHIS believes that current control measures (e.g., indexing of potatoes for viruses) are adequate to control any potential new virus that may arise in potatoes.

E. Potential Impact on Nontarget Organisms, Including Beneficial Organisms.

Consistent with its statutory authority and requirements under NEPA, APHIS evaluated the potential for these two lines to have damaging or toxic effects directly or indirectly on nontarget organisms, particularly those that are recognized as beneficial to agriculture and to those which are recognized as threatened or endangered in the United States. APHIS also considered potential impacts on other "nontarget" pests, since such impacts could have an impact on the potential for changes in agricultural practices.

There is no reason to believe that deleterious effects or significant impacts on nontarget organisms, including threatened and endangered species or beneficial organisms, would result from the NPTII conferring kanamycin resistance that was used as a selectable marker during development of Monsanto transgenic potato transformation events. This protein has been approved for human consumption by FDA (Internet address

http://vm.cfsan.fda.gov/~lrd/biotechm.html, see: Listing of final consultations under FDA's Biotechnology Policy). The application of kanamycin to these two potato lines when grown on commercial scale is highly unlikely and would require additional Federal government review.

There is no reason to believe that deleterious effects or significant impacts on nontarget organisms including threatened and endangered species or beneficial organisms, would result from the expression of PLRV *orf* 1 and 2. This protein is found in all PLRV-infected plants and there are no reports of this protein (or PLRV-infected plants) having any toxic effects (Matthews, 1991). EPA has granted these proteins an exemption for tolerance from FFDCA (http://www.epa.gov/fedrgstr/EPA-PEST/1997/August/Day-15/p21691.htm).

EPA has previous reviewed and approved the use of the plant-pesticide CryIIIA in several CPB-resistant potato plants. This review included analysis of toxicity to mammals, allergenicity, and environmental fate. Environmental fate data included avian, nontarget and beneficial insect, honeybee and nontarget organism. EPA determined that CryIIIA will not effect threatened and endangered species (http://www.epa.gov/fedrgstr/EPA-PEST/1995/May/Day-03/pr-243.html and the Pesticide fact sheet for Plant-Pesticide *Bacillus thuringiensis* CryIII(A) delta endotoxin and the genetic material necessary for its production in potato, Conditional Registration that is available upon request).

The development of resistance of the CPB to CryIIIA is an issue. A voluntary resistance management strategy has been adopted by Monsanto and EPA to delay the development of resistant insects (http://www.epa.gov/fedrgstr/EPA-PEST/1995/May/Day-03/pr-243.html and the Pesticide fact sheet for Plant-Pesticide *Bacillus thuringiensis* CryIII(A) delta endotoxin and the genetic material necessary for its production in potato, Conditional Registration that is available upon request).

Based on this analysis, APHIS concludes that there is unlikely to be any significant adverse impact on environment associated with the cultivation of CPB- and PLRV-resistant potatoes.

VI. CONCLUSION

APHIS has evaluated available information from the scientific literature and scientific community as well as data submitted by Monsanto that characterized CPB- and PLRV-resistant potatoes. After careful analysis, APHIS has identified no significant impact to the environment from issuance of a determination that CPB- and PLRV-resistant potatoes should no longer be regulated articles under APHIS regulations at 7 CFR Part 340.

APHIS has considered the foreseeable consequences of removing CPB- and PLRV-resistant potatoes from these regulations, and has reached the following conclusions:

1. CPB- and PLRV-resistant potatoes exhibit no plant pathogenic properties. Although pathogenic organisms were used in their development, these potato plants are not infected nor can they incite disease in other plants.

2. CPB- and PLRV-resistant potatoes are no more likely to become weeds than similar pest-resistant potatoes developed by traditional breeding techniques. Potatoes are not a weed pest in the U.S., and there is no reason to believe that resistance to CPB and PLRV would enable potatoes to become a weed.

3. Multiple reproductive barriers ensure that gene introgression from these CPB- and PLRV-resistant potatoes into wild or cultivated sexually-compatible plants are unlikely. Even in the unlikely event of gene introgression, this should not increase the weediness potential of resulting progeny or have an adverse impacts on biodiversity than similar pest-resistant potatoes developed by traditional breeding techniques.

4. Except for being pest resistant, these potatoes are substantially equivalent to nontransgenic tubers and therefore, APHIS can foresee no adverse impacts on raw or processed agricultural commodities.

5. CPB- and PLRV-resistant potatoes exhibit no significant potential to either harm organisms beneficial to the agricultural ecosystem, to have an adverse impact on the ability to control nontarget insect pests, or to harm threatened and endangered species.

APHIS concludes that CPB- and PLRV-resistant potatoes will be just as safe to grow as potatoes that are not subject to regulation under 7 CFR Part 340, and that there should be no significant impact on the human environment if CPB- and PLRV-resistant potatoes were no longer considered regulated articles under its regulations (7 CFR Part 340).

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APPENDIX I: RESPONSE TO MONSANTO COMPANY PETITION 97-204-01p FOR DETERMINATION OF NONREGULATED STATUS FOR COLORADO POTATO BEETLE- AND POTATO LEAF ROLL VIRUS-RESISTANT POTATO LINES

Prepared by
United States Department of Agriculture
Animal and Plant Health Inspection Service
Plant Protection and Quarantine

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I. SUMMARY

Based on a review of scientific data and literature, the Animal and Plant Health Inspection Service (APHIS) has determined that potato leaf roll (PLRV)-resistant and Colorado potato beetle (CPB) (*Leptinotarsa decemlineata*)-resistant cv. Russet Burbank potato events, RBMT21-129 and RBMT21-350 (hereafter referred to as CPB- and PLRV-resistant potatoes), do not represent a plant pest risk and are therefore not regulated articles under the regulations found at 7 CFR Part 340. Because of this determination, oversight under those regulations will no longer be required from APHIS for field testing, importation, or interstate movement of CPB- and PLRV-resistant potatoes or their progeny. This determination by APHIS has been made in response to a petition received from Monsanto Company (Monsanto) on July 23, 1997. The petition requested a determination from APHIS that the CPB- and PLRV-resistant potatoes do not present a plant pest risk and are therefore not regulated articles.

These potatoes have been developed as an alternative means of providing season-long control of the two damaging pest of potato crops, Colorado potato beetle, *Leptinotarsa decemlineata* (Say), and the virus potato leaf roll. The resistance genes conferring resistance to CPB and PLRV were introduced via genetic engineering techniques. These techniques enabled the developer to express in potato plants the gene *cryIIIA* from the soil bacterium *Bacillus thuringiensis* subsp. *tenebrionis* encoding a highly selective insecticidal delta-endotoxin crystalline protein, CryIIIA, the open reading frame (*orf*) 1 and 2 gene from PLRV that encodes a helicase and replicase, and the selectable marker gene encoding the enzyme neomycin phosphotransferase *(nptII)*. The *nptII* gene, isolated from a common human colon bacterium, *Escherichia coli*, encodes an enzyme that confers resistance to antibiotics kanamycin and neomycin used in the selection of transformed cells. All the genes were introduced via a well-characterized procedure that results in direct introduction of genes into plant genomes.

APHIS regulations at 7 CFR Part 340, which were promulgated pursuant to authority granted by the Federal Plant Pest Act (FPPA), as amended (7 U.S.C. 150aa-150jj) and the Plant Quarantine Act (PQA), as ammeded (7 U.S.C. 151-164a, 166-167) 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. Section 340.6 of the regulations, entitled, "Petition Process for Determination of Nonregulated Status," provides that a person may petition the agency to evaluate submitted data and determine that a particular regulated article does not present a plant pest risk and should no longer be regulated. If the agency determines that the regulated article does not present a risk of introduction or dissemination of a plant pest, the petition would be granted, thereby allowing for unregulated introduction of the article in question.

CPB- and PLRV-resistant potatoes have been considered "regulated articles" for field testing under Part 340 of the regulations, in part, because they have been engineered using components

from known plant pests. The vector system used to transfer the genes into the recipient potato was derived from the bacterial plant pathogen, *A. tumefaciens.* Also, certain noncoding regulatory sequences were derived from figwort mosaic virus (FMV) and *A. tumefaciens* and the *orf* 1 and 2 genes were isolated from the known plant pest PLRV.

Field testing of these potatoes has been done under APHIS oversight starting in late 1993 and continuing in 1998. All field trials were performed under conditions of reproductive confinement.

This determination is made based on an analysis that revealed that these CPB- and PLRV-resistant potatoes; exhibit no plant pathogenic properties; are no more likely to become a weed than similar pest-resistant potatoes developed by traditional breeding techniques; gene introgression from CPB- and PLRV-resistant potatoes into wild or cultivated sexually-compatible plants is unlikely, and such rare events should not increase the weediness potential of resulting progeny or have an adverse impacts on biodiversity than similar pest-resistant potatoes developed by traditional breeding techniques; are substantially equivalent to nontransgenic tubers and should have no adverse impacts on raw or processed agricultural commodities; and exhibit no significant potential to either harm organisms beneficial to the agricultural ecosystem or to have an adverse impact on the ability to control nontarget insect pests, or to harm threatened and endangered species.

II. BACKGROUND

A. APHIS regulatory authority

APHIS regulations at 7 CFR 340, which were promulgated pursuant to authority granted by the Federal Plant Pest Act (FPPA), as amended (7 U.S.C. 150aa-150jj) and the Plant Quarantine Act (PQA), as amended (7 U.S.C. 151-164a, 166-167), regulate the introduction (importation, interstate movement, or release into the environment) of certain genetically engineered organisms and products. Under this regulation, a genetically engineered organism is deemed a regulated article either 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 APHIS has reason to believe that the genetically engineered organism presents a plant pest risk.

Before the introduction of a regulated article, a person is required under Section 340.0 of the regulations to either (1) notify APHIS in accordance with Section 340.3 or (2) obtain a permit in accordance with Section 340.4. Introduction under notification (Section 340.3) requires that the introduction meets specified eligibility criteria and performance standards. The eligibility criteria impose limitations on the types of genetic modifications that qualify for notification, and the performance standards impose limitations on how the introduction may be conducted. Under Section 340.4, a permit is granted for a field trial when APHIS has determined that the conduct of the field trial, under the conditions specified by the applicant or stipulated by APHIS, does not pose a plant pest risk.

An organism is not subject to the regulatory requirements of 7 CFR Part 340 when it is demonstrated not to present a plant pest risk. Section 340.6 of the regulations, entitled "Petition Process for Determination of Nonregulated Status," provides that a person may petition the agency to evaluate submitted data and determine that a particular regulated article does not present a plant pest risk and should no longer be regulated. If the agency determines that the regulated article does not present a risk of introduction or dissemination of a plant pest, the petition will be granted, thereby allowing for unregulated introduction of the article in question. A petition may be granted in whole or in part.

B. EPA and FDA regulatory authority

The CPB- and PLRV-resistant potato lines are currently subject to regulations administered by the EPA or the FDA as described in the Environmental Assessment. FDA has completed the consultation procedure.

C. Rationale for Developing CPB- and PLRV-resistant Potatoes

Colorado potato beetle is the most damaging pest of potatoes (National Potato Council, 1992). If CPB is not controlled yield reductions can approach 85%. Nonchemical methods to control CPB are available but are not widely used because of lack of effectiveness.

There are no chemicals (antivirals) that are effective to control plant viruses. PLRV is transmitted from plant to plant by aphids. Leaves of infected plants often show pallor, and in some cultivar reddening of the tips of leaves, which may become rolled and assume an erect habitat. Net necrosis is a death of the vascular tissue in the tuber that results in a discoloration of the tuber. Discolored tubers are not saleable. The goal of Monsanto's virus resistance is to reduce net necrosis not necessarily eliminate leave symptom development. To control PLRV, growers used certified seeds (tuber seed pieces). Certified seed must have less than 1% viral infection. When aphid populations are low, this low level of viral infection is not a significant concern. However, when aphid populations are high, the low level infection in the certified potatoes can be sufficient inoculum to result in nearly all the plants becoming infected by the end of the season. When aphid populations are high, insecticides are usually applied. Since many of the chemical insecticides have broad spectrum, most insecticides applied to potatoes would kill both CPB and the aphid vectors of PLRV. Monsanto estimates that nearly 2 million out of the 2.5 million pounds of chemical insecticides that were applied to potatoes were targeted to CPB and aphids.

III. RESPONSE TO COMMENTS

APHIS received 17 comments, all in favor, for this petition.

IV. ANALYSIS OF THE PROPERTIES OF CPB- and PLRV-resistant POTATOES

A. The introduced genes, their products, and the added regulatory sequences controlling their expression do not present a plant pest risk in CPB- and PLRV-resistant potatoes.

The two CPB- and PLRV-resistant potato lines were produced using an *Agrobacterium*-meditated transformation protocol to transform (by two independent transformations events) Russet Burbank. This technique is well studied (Klee and Rogers, 1989; and Zambryski, 1988). Transformed material was treated with chemicals and checked to ensure that *Agrobacterium* was not present.

The gene conferring CPB resistance designated *cryIIIA* (Höfte and Whitely, 1989) was isolated from *B. thuringiensis* subsp. *tenebrionis* BI 256-82 (*Btt*). Subspecies of the gram-positive soil bacterium *B. thuringiensis* are characterized by their ability to produce inclusions of crystalline proteins (delta-endotoxins) with highly specific insecticidal activity. The native gene encodes both a full length, 73 kD protein and a smaller 68 kD version of this protein (*Btt* band 3 protein) that results from the use of a downstream translational initiation site (McPherson et al., 1988, Perlak et al., 1993). Both proteins exhibit the same selective insecticidal activity against a narrow range of coleopteran insects (MacIntosh et al., 1990, McPherson et al, 1988). Upon ingestion of these proteins by susceptible insects, feeding is inhibited with disruption of the midgut epithelium, which eventually results in death (Slaney, et al., 1992). The gene encoding the *Btt* band 3 protein was modified for increased plant expression by the use of plant-preferred amino acid codons, but the resulting amino acid sequence remains unchanged.

In 1985 Sanford and Johnston proposed that if one understands the molecular interaction involved in the functioning of the pathogen, then mechanisms can be devised for interfering with them. The usefulness of this concept, called pathogen-derived resistance, has been best demonstrated with plant viruses. Plant viral genes have been expressed in transgenic plants, and those plants have generally been resistant to infection to the virus that provided the gene. The genes that have provided the best resistance have been the viral coat protein (Powell-Abel et al., 1986) and replicase (Golemboski et al., 1990). Monsanto has based their resistance strategy to PLRV by expressing the *orf* 1 and 2 that encodes the helicase/replicase in potatoes.

Luteoviruses are phloem-limited, spherical viruses that are only transmitted by certain aphids in a circulative, nonpropagative manner. The viral genome consists of single RNA molecule about 5,500 bases long and it codes for five or six proteins. The gene that encodes the resistance phenotype for PLRV in these two lines are the *orf* (Open Reading Frame) 1 and 2 that encode the PLRV helicase and replicase. Helicase enzyme is thought to be required to separate (break the hydrogen bonds) the double stranded viral RNA intermediate during viral RNA synthesis. The replicase gene is encodes the enzymatic function involved in ribonucleotide polymerization. These two proteins are the only viral proteins absolutely required for the virus multiplication in

protoplasts (Reutenauer et al., 1993). PLRV helicase and replicase are produced in a novel fashion. Unlike most plant proteins where one protein is produced from one messenger RNA, a single viral messenger RNA is translated to give rise to the two viral proteins. The helicase (*orf* 1) is translated in frame from the start signal (AUG) near the 5' end of the RNA and translation stops approximately two-thirds of the way from the start signal. The replicase (*orf* 2) synthesis starts with the same start signal as *orf* 1 but after half of the helicase gene is translated, the ribosome shifts backward (-1) on the RNA so that the RNA is read in a different triplet group, and now the complete RNA is translated. This frameshift occurs less than 10 percent of the time. The result of the frameshifting is two-fold (Brault and Miller, 1992). First, *orf* 1 protein production greatly exceeds *orf* 2 protein production. Second, the *orf* 2 replicase is a chimeric protein with small section of the helicase protein coupled to the replicase protein. Frameshifting for the synthesis of helicase-replicase complexes in viruses is common in several virus families (Atkins et al., 1990). There may be other yet to be identified replication functions for *orf*s 1 and 2 besides helicase and replicase. A likely candidate is the genome-linked protein (VP_g) which is believed to prime RNA synthesis for the replicase.

Luteoviruses are divided into two major subgroups. The coat proteins of luteoviruses are remarkably similar to each other. In contrast, subgroup I has replicase genes that are more closely related to those of dianthoviruses, umbraviruses, and carmoviruses than they are to subgroup II luteoviruses. The replicases of subgroup II, which includes PLRV are more closely related to those of sobemoviruses. Suprisingly, the replicase genes of these two luteoviral subgroups are as different as two such related genes can be (Miller et al., 1997).

CPB- and PLRV-resistant potatoes have also been transformed a selectable marker that enables identification and selection of the transformed plant cells during tissue culture. The neomycin phosphotransferase gene (nptII)(Beck et al., 1982, Jorgensen et al., 1979), isolated from a common human colon bacterium, *Escherichia coli*, encodes an enzyme that confers resistance to antibiotics kanamycin or neomycin used in the selection of transformed cells. NPTII inactivates kanamycin by phosphorylating it. The presence of nptII gene does not result in the presence in the production of the antibiotic kanamycin. The presence of nptII gene in these two transformation events does not mean that kanamycin will be used in the cultivation of these potatoes.

Data provided by Monsanto demonstrate that lines RBMT21-129 and RBMT21-350 contain the following sequences:

> Npt II gene whose transcription is directed by the nopaline synthase promoter (Fraley et al., 1983) and whose termination sequences are also derived from nopaline synthase gene (Depicker et al., 1982; Bevan et al., 1983). Both regulatory sequences are from *A. tumefaciens.*
>
> PLRV *orf* 1 and 2 and associated intergenic region transcription is directed by the figwort mosaic 35S promoter (Richins et al., 1987) coupled with the soybean heat shock protein

leader sequence (Rascke et al., 1988) and whose termination sequences are derived from the pea ribulose-1,5-bisphosphate carboxylase small subunit gene (Coruzzi et al., 1984).

CryIIIA gene whose transcription is directed by the *Arabidopsis thaliana* ribulose-1,5-bisphosphate carboxylase small subunit gene (Almedia et al., 1989; Wong et al., 1992) and whose termination sequences are derived from nopaline synthase.

Also, the left and right border sequences from *A. tumefaciens* are found in all 3 lines. Data was presented that demonstrated that sequences outside the border sequences including *oriV* and *aad* gene that confers streptomycin resistance are **not** present in any of the two lines.

Although the transformation process used the plant pathogen, *A. tumefaciens* (the causal agent of crown gall disease), the genes that cause crown gall disease were removed, and therefore the potato plant does not develop crown gall disease. Once inserted into the chromosome of the transformed plant, the introduced genes are maintained in the same manner as any other genes. Some regulatory sequences were derived from known plant pests these sequences can not incite disease.

During field testing, the CPB- and PLRV-resistant potatoes exhibited the typical agronomic characteristics of the recipient Russet Burbank, with the exception of the desired CPB- and PLRV-resistant phenotype.

Development of Viral Symptoms on Some Transgenic Plants

In the revised submission (dated October 17, 1997), on page 50, section C, entitled "Impact of potato leaf roll virus resistance", APHIS noted that a small percentage of PLRV-inoculated transgenic plants developed symptoms. On November 24, 1997, Monsanto submitted, at APHIS' request, additional data clarifying the previously submitted data. The revised data showed that, depending on the year, the location of the test, and which transformation event was inoculated, upwards of 18% of the plants showed developed leaf roll symptoms on their leaves. In 1996 under field conditions with natural infections, 60% of the control Russet Burbank developed symptoms, while only 1.4% event RBMT21-129 plants showed symptoms. The other lines tested showed no symptoms under these conditions.

APHIS then asked Monsanto to address whether the development of symptoms in these plants was a result of the challenge virus overcoming the resistance. On July 10, 1998, Monsanto submitted data supporting their contention that a resistance breaking strain had not developed, and that the failure the *orf* 1 and 2 transgene to protect the plant is probably due to high inoculum pressure or weak expression of the *orf* 1 and 2 gene. Monsanto also demonstrated that in the symptomatic transgenic plants, the transgenes had not been lost. Monsanto states that the goal of the engineering was to block net necrosis of the tubers, the viral symptom that has the major impact on the salability of the tubers.

The Use of CPB- and PLRV-resistant Potatoes Should Not Increase the Likelihood of the Emergence of New Plant Viruses

A. Recombination

Recombination is defined as an exchange of nucleotide sequences between two nucleic acid molecules. Recombination between viral genomes results in heritable, permanent change. The persistence of a recombined viral genome will depend upon its fitness with respect to its ability to replicate within the original host cell, its ability to replicate in the presence of parental viruses, its ability to spread systemically within the host, or its successful transmission to other host plants.

Factors that influence recombination rates and detection of a viable recombinant include sequence and structural similarity between the nucleic acid molecules, subcellular location and concentration of the nucleic acids, and the number of recombinational events required to form a viable recombinant viral genome (Lai, 1992). The frequency of recombination between two naturally occurring viruses or two viral strains in field-grown plants in the absence of selection pressure has not been determined (Henry et al., 1995) and is difficult or impossible to measure meaningfully. In transgenic plants expressing sequences derived from either a DNA virus (Schoelz and Wintermantel, 1993) or RNA virus (Greene and Allison, 1994), it has been demonstrated that recombination between a viral transgene and a defective challenge virus can restore an functional, infective virus. These results demonstrate that recombinational events occur in plants expressing viral sequences when inoculated with defective viruses but say little about what happens with nondefective viruses replicating in resistant transgenic plants.

Recombination is hypothesized as an important mechanism for virus change over evolutionary time frames and may have been quite frequent over this time (Simon and Bujarski, 1994). Recently, the nucleotide sequences of numerous viral strains from many of the known genera have been published. Sequencing data have shown that certain genes in quite different taxa probably arose from recombinational events (Gibbs, 1995; Gibbs and Cooper, 1995). Miller *et al.* (1997) noted that based on nucleotide sequence homology two major subgroups of luteoviruses probably arose via recombination. Subgroup I, which includes BYDV strains PAV and MAV and SDV, has replicase genes that are more closely related to those of dianthoviruses, umbraviruses, and carmoviruses than they are to subgroup II luteoviruses. The replicases of subgroup II, which includes PLRV, BYDV strains RPV and RMV, BWYV are more closely related to those of sobemoviruses. The replicase genes of these two subgroups are as different as two such related genes can be. Currently, it is not possible to determine whether these recombinational events occurred, for example, since the development of modern agricultural cropping practices or in much longer time frames.

These two lines, like most transgenic plants field tested to date in the U.S. under APHIS oversight, contain viral derived transgenes from viruses that regularly infect the host plant,

because damage by those viruses poses the most constant potential for loss in the crop species. Sequences from those viruses, when available for recombination, would be unlikely to pose the potential for generating novel recombinants in comparison with natural mixed infections in the recipient plant.

The use of virus-resistant transgenic plants in agriculture highlights the following three questions regarding recombination.

1) Is the viral transgene produced in the same cells that it is normally found in during viral infection?

One novel aspect of this engineering as compared to other previously approved virus-resistant transgenic plants is that the transgene is expressed in mesophyll cells where the virus does not replicate to any significant degree. Thus, does the expression of the PLRV *orf* 1 and 2 in more cells than it is found in during viral infection of nontransgenic plants raise the likelihood of recombination or a recombinant virus arising? Although luteoviruses are often called phloem limited, a large number of companion cells and occasionally parenchyma cells are infected (van deb Heuvel *et al.* 1985). The most likely viruses to recombine with viral transgene are those viruses that naturally infect potatoes. The most widely prevalent viruses in the U.S. that infect potatoes are: potato aucuba mosaic potexvirus, potato virus A potyvirus, potato virus M carlavirus, potato virus S carlavirus, potato virus X potexvirus, potato virus Y potyvirus, and potato yellow dwarf nucleorhabdovirus (from APHIS' Widely Prevalent Viruses by State, 1998). Homologous recombination, i.e. recombination between sequences that show homology, is more likely to occur with viruses in the same taxa because they share sequence homology. No other luteovirus routinely infects potatoes in the U.S. Related viruses in luteovirus subgroup II include BYDV strains RPV and RMV, BWYV, all of which do not infect potatoes (Hooker, 1981). Subgroup I luteoviruses have replicase genes as different as two such related genes can be (Miller et al., 1997), thus recombination is unlikely. For potato viruses that are in different taxa, potyviruses, potexviruses, carlaviruses, and nucleorhabdovirus all replicate in mesophyll, companion, and parenchyma cells (Matthews, 1991; Lawson *et al.* 1971; Foster 1992).

APHIS believes that expression of PLRV *orf* 1 and 2 in the mesophyll tissues might raise the likelihood of recombination because more cells contain PLRV *orf* 1 and 2 sequences but not the likelihood of a new recombinant virus arising because of selection pressure (see section C below).

2) What factors may affect the rate of recombination, and will that rate be proportional to the concentrations to transgene RNA molecules?

With respect to amount of transgene RNA available for recombinational event, Monsanto has provided data to support that the concentration of transgene RNA in these lines is approximately five to ten-fold less than the amount viral mRNA in viral-infected nontransgenic plants. APHIS notes a discussion among virologists on this issue, "The implications of these low expression

levels for recombination are not clear. Even assuming that the higher concentration of transgene RNA the greater the chance for recombination, we do not know what a meaningful range is; what are low and high concentrations of transgene transcript relative to unacceptable recombination rates?" (AIBS, 1995). APHIS believes that the significantly lower concentration of transgene RNA in these lines is reassuring considering recombination has not been detected in transgenic plants with nondefective viruses and the other points raised in this section.

3) Are any recombinants thus formed likely to be successful in competition with parental viruses?

If a recombinant virus is formed in a cell (either in a transgenic plant or during a mixed infection) will that recombinant participate in the replication process in that cell, move systemically in the plant, or cause a new disease? The vast majority of progeny viruses do not apparently function in the replication process. For many viruses, the newly synthsized RNA is rapidly encapsidated by coat protein. Then, viral RNA synthesis in the cell ceases or declines to undetectable levels. Unless virus is transmitted to another plant by a vector or via progeny plants, the virions are degraded when the plant cell dies (Matthews, 1991). The likelihood of a recombinant becoming established depends on many factors, including: its competitiveness with infecting virus and other viruses that naturally infect the plant and by all the additional factors that may affect selection pressure (e.g., temperature, vectors, host plants). Thus, to predict the probability of development of new virus disease resulting from recombination of two viruses or between a virus and a viral derived transgene, requires a considerable level of understanding of the population biology of viruses in cells and virus movement within plants, and a better understanding of the mechanisms of how viruses cause disease.

In conclusion, there is the possibility that recombination between a transgene and virus could occur, but it is likely that the recombinant virus either would not be viable or the recombinant virus would be viable but not competitive with the wild type virus. Although much of the discussion on the risk of using viral-derived transgenes has focused on the risk of recombination/recombinant virus, there is no persuasive evidence that recombinant viruses pose a greater risk to plants than any of new virus or viral strain that is identified each year in potatoes.

B. Transcapsidation

When a single plant cell is simultaneously infected by two different strains of a virus (or two viruses), it may be possible for the genome of one virus to become encapsidated by coat protein of the second virus. If the virus is encapsidated by only one of the coat proteins, it is termed genomic masking or transcapsidation Since the resistance gene is replicase, transcapsidation is not an issue.

C. Synergy

Occasionally, when two viruses simultaneously **naturally** infect a plant, the symptoms can be more severe than when either of the viruses infects the plant singly. This phenomenon is called synergy (Matthews, 1991). Synergistic infections can often result in severely diseased, unmarketable crops. Synergy was first described and is best studied with potato (potex)virus X (PVX) and potato (poty)virus Y (PVY). There are no reports of PLRV causing synergistic interaction with any other plant viruses (OECD, 1996). Monsanto did not observe synergistic symptoms during field testing of these lines. APHIS believes that the appearance of synergistic symptoms with these two lines is highly unlikely. APHIS believes that symptoms caused by synergistic viral interactions are an agronomic problem (not an environmental issue) in that the yield of the plant is reduced or the symptoms so severe that the plant cannot be sold. A similar conclusion regarding synergy being an agronomic problem was reached by scientists in a public meeting that discussed virus resistant transgenic plants (AIBS, 1995).

D. Satellites and host RNA polymerases

In a review article, Miller *et al.* (1997) raised three questions regarding the risks of commercial use of luteovirus resistant plants. Below is APHIS' response to these questions.

Issue 1. Satellite RNAs and ST9a RNA have been identified with certain luteoviruses. Could satellite RNAs be replicated by PLRV *orf* 1 and 2 transgene? What potential impacts could be envisioned?

Some viral infections are also associated with the production of satellite RNAs or satellite viruses. Satellite RNAs depend on a specific virus (called helper virus) for the replication enzymes needed to replicate their own RNA, the RNAs are usually smaller in size than their helper viral genome, and have no significant sequence homology to the helper virus genome. In certain host plants, the presence of a satellite RNA can affect disease symptoms (Matthews, 1991). In satellite viruses, the satellite codes for its own coat protein,whereas satellite RNAs are packaged in the coat protein of the helper virus

ST9a RNA is an RNA associated with aphid-transmissible beet western yellows luteovirus (BWYV). This RNA enhances the replication of BWYV and causes more severe symptoms in infected plants. ST9a RNA encodes its own replicase and can replicate independently in protoplasts (Passmore et al., 1993; Chin et al., 1993). It depends on BWYV for its coat and movement proteins. ST9a RNA is the only known luteoviral satellite present in the U.S. (Bryce Falk, University of California, Davis, personal communication).

a. There are no confirmed reports that BWYV naturally infects potatoes (Barker, 1986). Thus, it would be unlikely that ST9a RNA would ever infect transgenic potatoes.

b. Even if ST9a RNA (and BWYV) did infect a transgenic plant, the ST9a RNA has its own replicase. There is no reason to believe that there would be any advantage for the satellite to

recombine with the replicase transgene. No known RNA virus has two replicase genes. Even in the unlikely event that ST9a RNA was amplified by transgene replicase, the encapsidation and movement functions are still lacking (provided under natural conditions by BWYV), and thus any impact would be limited to a few initially infected cells.

c. No satellites have been reported to be associated with potato leaf roll luteovirus (PLRV). In the unlikely event a satellite infected the transgenic plants and was amplified by the replicase transgene, the satellite would not be able to move systemically in the plant without other helper virus components. For example, in the absence of coat protein, the satellite RNAs could not be encapsidated and effectively transmitted in the field, because both of these functions are provided by the helper virus. Although satellites can either attenuate or intensify symptoms, symptom development in the plant would be mainly of an agronomic problem not environmental impact.

Issue 2. Could host cell RNAs be transcribed by the replicase transgene? Is there evidence that this could occur? What potential impacts could be envisioned?

One of the quintessential characteristics of viral replicases is their specificity toward the RNA that they replicate (Dorssers et al., 1984; Miller et al., 1986). Even if there was amplification of a host RNA, there is no evidence that it would result in any visible symptoms, that it could move from cell-to-cell, or that it could move from plant to plant.

Issue 3. All plants have an endogenous RNA-dependent RNA polymerase. What is likelihood that this enzyme could make the complementary minus strand of the replicase transgene? If the minus strand is produced, then the sequences that encode subgenomic promoter sequences would be produced.

Based on the studies of this plant enzyme and viral replicases, there is no evidence to support a hypothesis that the complementary minus strand of the transgene mRNA could be synthesized by the host plant RNA polymerase (Dorssers et al., 1984; Miller et al., 1985; Fraenkel-Conrat, 1986).

In conclusion, based on the above points APHIS believes that because the viral transgene is derived from virus that naturally infects the potato host, is produced in less concentration than during natural infections, and if a recombinant was formed would have to be competitive with other potato- infecting viruses, the likelihood of novel interactions and formation of recombinant virus is no more likely than its occurrence during mixed infections. Although the expression of *orf* 1 and 2 in mesophyll might raise the frequency of recombination, APHIS believes that the recombinant would not be competitive with other potato viruses. APHIS believes that even if a recombinant virus did occur that this virus could be managed just like the numerous new viruses that are detected every year in the United States.

The AIBS report to USDA (1995) concludes by stating, "With or without the use of transgenic plants, new plant virus diseases will develop that will require attention." APHIS concurs with their statements.

D. CPB- and PLRV-resistant potatoes have no significant potential to become successful weeds.

It is unlikely that expression of the CPB insect control protein in the CPB- and PLRV-resistant potatoes will provide a competitive advantage sufficient to cause these to be more "weedy" than standard or other potato cultivars. None of the characteristics of weeds described by Baker (1965) involved resistance or susceptibility to insects. Resistance to CPB does not seem to be a critical factor determining weediness in Solanaceous species. Some *Solanum* species listed as common weeds in the U.S., i.e., the nightshades, are not resistant to CPB, and in fact, some are common hosts, but they do have many of the other "weedy" characteristics described by Baker (Muenscher, 1955, USDA, 1971). Although no cultivated potato varieties are available that are resistant to CPB, varieties have been developed that are resistant to other insects. For example, the variety "Norchip" is resistant to flea beetle (Thompson, 1987) and is not known to be more "weedy" than the variety from which it was developed. The database of the USDA Germplasm Resources Information Network (GRIN, 1994) contains accessions of at least 15 different species in the genus *Solanum* L., subgenus *Potato*, section *Petota* reputed to have resistance to CPB and collected in countries (i.e., Costa Rica, Guatemala, Mexico, and the United States) where CPB is listed as a pest (C.A.B. International, 1991). None of these species is listed as a serious, principal or common weed in these countries by a leading weed compendium (Holm et al., 1991).

Resistance or tolerance genes have been identified in *Solanum sp.* Resistance in modern cultivars can be traced back to hybrids from *S. demissum* - ssp. *andigena* - ssp. *tuberosum*. Other examples include the W races, hybrids that include MPI 44.335 (which includes clone MPI 19268), an ancestor of many leafroll resistant Dutch cultivars. A high level of resistance is also inherited by *S. acaule* x ssp. *tuberosum* - back cross hybrids e.g. MPI44.1016/10. The degree of leafroll resistance in European cultivars has been described. However, some PLRV resistant cultivars do have reduced flowering and processing characteristics that limit their usefulness (Ross, 1986). In addition, somaclonal variants of Russet Burbank lines have been isolated and shown to be resistant to PLRV during field testing (Kawchuk, 1997). Thus, the introduction of Monsanto's virus resistant lines should not increase the weediness potential of potatoes than do plants that shown resistance by breeding or somaclonal variation.

Based on evaluation of the available literature and data submitted by Monsanto, APHIS concludes that these CPB- and PLRV-resistant potatoes are no more likely than other traditionally developed pest-resistant potatoes to present a plant pest risk as weeds.

E. Multiple barriers insure that gene introgression from CPB- and PLRV-resistant potatoes into wild or cultivated sexually-compatible plants is extremely unlikely, and such

rare events should not increase the weediness potential of resulting progeny or adversely impact biodiversity.

APHIS first evaluated the potential for gene flow from CPB- and PLRV-resistant potatoes to other cultivated and wild relatives. The kanamycin resistance trait used as a selectable markers in these potatoes was not considered in this analysis, because there is no selection pressure for this trait in plants in nature (i.e., kanamycin will not applied to field crops).

Since these two transgenic lines, like the untransformed parent line, Russet Burbank, are male sterile, the likelihood of pollen forming is highly unlikely. Thus, movement of these resistance genes to other sexually compatible species is also highly unlikely. In the remote chance that male-fertile transgenic progeny are produced from CPB- and PLRV-resistant potatoes as a result of introgression into another potato cultivar, APHIS evaluated the potential for gene transfer from such progeny to wild or free-living sexually-compatible species occurring in the United States and centers of origin for potatoes.

Besides geographical barriers, other barriers exist that have prevent hybridization of wild species directly with cultivated *S. tuberosum* under natural field conditions. These barriers include multiple ploidy levels, incompatibility, and endosperm balance numbers (EBN) (i.e., the ratio of maternal to paternal genomes in the endosperm) which when unequal, can lead to endosperm failure and embryo abortion. Species with identical EBNs are usually crossable; however, these three wild species have EBNs of 1 or 2, and are therefore incompatible with the EBN of 4 for *S. tuberosum*.

Tuber-forming *Solanum* species, including *Solanum tuberosum*, are unsuccessful in forming natural hybrids with the native or introduced weedy *Solanum* species in the U.S. that do not form tubers, including bitter nightshade (*S. dulcamara*), silverleaf nightshade (*S. elaeagnifolium*), black nightshade (*S. nigrum*), hairy nightshade (*S. sarrachoides*), cutleaf nightshade (*S. triflorum*), buffalobur (*S. rostratum*), and turkeyberry (*S. torvum*) (Love, 1994). Successful gene introgression into tuber-bearing *Solanum* species occurring in the United States is also virtually excluded. Only three related tuber-bearing *Solanum* species (i.e. *S. jamesii*, *S. fendleri*, and *S. pinnatisectum*) have been well documented to occur in the United States. Geographical isolation reduces the chances for natural hybridization of these species with *S. tuberosum*. *S. pinnatisectum* is limited to a small area in Arizona, and the other two species have been found in Arizona, Colorado, New Mexico, and Texas, with populations of *S. jamesii* also found in Nebraska and Utah. All of these species are native to dry, forested areas above 1600 m in elevation, although *S. fendleri* and *S. jamesii* have been observed growing in areas of potato production or around cultivated fields. Even though geographical isolation is not a complete hybridization barrier for these two species, no natural hybrids have ever been observed between these species and cultivated potatoes in the U.S. This also may be a result of different ploidy levels between *S. tuberosum* (4x) and *S. jamesii* and *S. pinnatisectum*, which are both diploid (2x).

This Determination does not carry with it any foreign safety presumption, because our authority extends to the borders of the United States and its territories and possessions. Questions have been raised by ecologists regarding the potential impacts associated with the cultivation of genetically engineered crops near their centers of diversity. Therefore, the following analysis is provided to address those potential impacts.

CPB- and PLRV-resistant potatoes are likely to be cultivated only where CPB is a serious pest and in environments to which it is suited. CPB is currently distributed widely in the U.S., southern Canada, Europe, Asia, Libya, Costa Rica, Cuba, Guatemala, and Mexico (C.A.B. International, 1991). Of these areas, central Mexico is also listed as a center of diversity for potatoes (Hawkes, 1990). Other known centers of diversity include Peru, Bolivia, and northwest Argentina. PLRV is a pest wherever commercial potatoes are grown (Hooker, 1981).

Hanneman (1994) thoroughly evaluated the potential for gene exchange between cultivated *S. tuberosum* (4x and 4EBN) and wild relatives in the Central and North American center of diversity and has provided a framework for evaluating potential impacts associated with introgression of transgenes from genetically engineered potatoes into wild relatives. He concluded that there is little threat of introduction of genes into the two tuber-bearing wild *Solanum* species (*S. longiconicum* and *S. woodsonii*) occurring in Costa Rica because of differences in their habitats (humid pine forests and clearings or mountains) and probable differences in EBN. Mexico has the greatest number of wild species known in North and Central America, and many species native to Mexico also exist in Guatemala. Introgression into many of these species would also be inhibited by EBN incompatibility. The possibility exists for introgression into 4x(4EBN) wild or native cultivated species, and wild species with 6x (or 5x)(4EBN), or through unreduced (2n) gametes of wild species with 2x(2EBN) and 4x(2EBN). In the latter case, unreduced gametes occur at relatively low frequencies; therefore, the chance for successful hybridization of these with CPB- and PLRV-resistant potatoes is low, and continued introgression into those species would also require unreduced gametes.

Of the other wild species with known (or anticipated) EBNs of 4, only *S. demissum* (6x), *S. x edinense* ssp. *salamanii* (5x) and *S. x semidemissum* (5x) (all classified in the *Solanum* series Demissa) have been found in or on borders of potato fields. These species are not listed as serious, principal or common weeds in Mexico by Holm *et al.* (1991), even though they are described as weeds by Hanneman (1994). *S. demissum* is found predominantly at high elevations in coniferous forests (Correll, 1962). *S. demissum* is reported to have poisonous components (glycoalkaloids) in the leaves that provide moderate resistance to CPB (Correll, 1962; Flanders et al., 1992). The GRIN Database (1994) lists 15 accessions of *S. demissum* reputed to have some resistance to the CPB. Hybrids between the hexaploid (6x) species in the series Demissa and 4x cultivated species have occurred, resulting in the pentaploid (5x) species as described above (Hanneman, 1994). Therefore, it is possible that some of these hybrids may already have some resistance to the CPB.

Local *S. tuberosum* ssp. *andigena* cultivars are cultivated in Costa Rica, Mexico and Guatemala, and they are capable of forming hybrids with conventionally bred potato cultivars because of their compatible ploidy and EBN (4x and 4EBN). But because they are generally cultivated in mountainous regions, and commercial *S. tuberosum* are generally cultivated at lower elevations, significant introgression into these local cultivars is unlikely. Introgression in all of these cases would be further limited by those barriers described previously.

APHIS has concluded that the possibility for introgression of Monsanto CPB- and PLRV-resistant potato germplasm into the wild and local cultivars of *Solanum* species in the Central American center of potato diversity is remote, and therefore the impact (if any) would be minimal. CPB-resistance is unlikely to provide a selective advantage to many of the wild *Solanum* species and *S. tuberosum* ssp. *andigena* cultivars grown in mountainous regions because *Leptinotarsa* species such as *Leptinotarsa decemlineata* (CPB) generally occur at lower altitudes (Flanders et al., 1992). CPB-resistance would also be unlikely to provide a selective advantage to native or commercial potato cultivars, because although the CPB is listed as a pest in this area, it is not a significant pest of cultivated potatoes. CPB originated in Mexico, and the populations there prefer weedy Solanaceous species, such as *S. rostratum* and *S. angustifolium*, instead of potatoes as hosts (Logan and Lu, 1993).

The impact of cultivation of CPB- and PLRV-resistant potatoes on the genetic diversity of wild tuber-bearing *Solanum* populations is likely to be comparable to that from these other nontransgenic varieties or cultivars that contain resistance or tolerance to CPB or PLRV.

F. Composition, quality and characteristics of CPB- and PLRV-resistant potato tubers indicate that there should be no adverse impacts on raw or processed agricultural commodities.

Monsanto reported no significant changes in tubers that would affect raw or processed potatoes. APHIS believes that the modifications for pest resistance should not affect this commodity in any significant manner.

G. CPB- and PLRV-resistant potatoes exhibit no significant potential to either harm organisms beneficial to the agricultural ecosystem or to have an adverse impact on the ability to control nontarget insect pests.

Consistent with its statutory authority and requirements under NEPA, APHIS evaluated the potential for these two transformation events to have damaging or toxic effects directly or indirectly on nontarget organisms, particularly those that are recognized as beneficial to agriculture and to those which are recognized as threatened or endangered in the United States. APHIS also considered potential impacts on other "nontarget" pests, since such impacts could have an impact on the potential for changes in agricultural practices.

There is no reason to believe that deleterious effects or significant impacts on nontarget organisms, including threatened and endangered species or beneficial organisms, would result from the expression of PLRV *orf* 1 and 2. This protein is found in all PLRV-infected plants and there are no reports of this protein (or PLRV-infected plants) having any toxic effects (Matthews, 1991). EPA has granted these proteins an exemption for tolerance from FFDCA (http://www.epa.gov/fedrgstr/EPA-PEST/1997/August/Day-15/p21691.htm).

The use of delta-endotoxins from *B. thuriengínsis* has been reviewed by EPA and they have approved the use of the plant-pesticide CryIIIA in several CPB- resistant potato plants. This review included analysis of toxicity to mammals, allergenicity, and environmental fate. Environmental fate data included avian, nontarget and beneficial insect, honeybee and nontarget organism. EPA determined that CryIIIA will not effect threatened and endangered species (http://www.epa.gov/fedrgstr/EPA-PEST/1995/May/Day-03/pr-243.html and the Pesticide Fact Sheet for Plant-Pesticide *Bacillus thuringiensis* CryIII(A) delta endotoxin and the genetic material necessary for its production in potato, Conditional Registration that is available upon request).

The development of resistance of the CPB to CryIIIA is an issue. A voluntary resistance management strategy has been adopted by Monsanto and EPA to delay the development of resistant insects (http://www.epa.gov/fedrgstr/EPA-PEST/1995/May/Day-03/pr-243.html and the Pesticide Fact Sheet for Plant-Pesticide *Bacillus thuringiensis* CryIII(A) delta endotoxin and the genetic material necessary for its production in potato, Conditional Registration that is available upon request).

There is no reason to believe that deleterious effects or significant impacts on nontarget organisms, including threatened and endangered species or beneficial organisms, would result from the NPTII conferring kanamycin resistance that was used as a selectable marker during development of Monsanto transgenic potato transformation events. The application of kanamycin to these two transformation events when grown on commercial scale is highly unlikely and would require additional Federal government safety review.

APHIS would like to note that NPTII has been approved for human consumption by FDA (Internet address http://vm.cfsan.fda.gov/~lrd/biotechm.html, see: Listing of final consultations under FDA's Biotechnology Policy).

APHIS concludes that CPB- and PLRV-resistant potatoes exhibit no significant potential to adversely impact organisms beneficial to plants or agriculture or to adversely impact the ability to control nontarget insect pests of agriculture.

V. CONCLUSION

APHIS has determined that CPB- and PLRV-resistant potatoes that have previously been field tested under permit, will no longer be considered regulated articles under APHIS regulations at 7 CFR Part 340. Permits or notifications acknowledged under those regulations will no longer be required from APHIS for field testing, importation, or interstate movement of those CPB- and PLRV-resistant potatoes or their progeny. (Importation of CPB- and PLRV-resistant potatoes [and nursery stock or seeds capable of propagation] is still, however, subject to the restrictions found in the Foreign Quarantine Notice regulations at 7 CFR Part 319). This determination has been made based on data collected from these trials, laboratory analyses and literature references presented herein which demonstrate the following: exhibit no plant pathogenic properties; are no more likely to become a weed than similar pest-resistant potatoes developed by traditional breeding techniques; gene introgression from CPB- and PLRV-resistant potatoes into wild or cultivated sexually-compatible plants is unlikely, and such rare events should not increase the weediness potential of resulting progeny or have an adverse impacts on biodiversity than similar pest-resistant potatoes developed by traditional breeding techniques; are substantially equivalent to nontransgenic tubers and should have no adverse impacts on raw or processed agricultural commodities; and exhibit no significant potential to either harm organisms beneficial to the agricultural ecosystem or to have an adverse impact on the ability to control nontarget insect pests, or to harm threatened and endangered species.

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