Notices

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[Docket No. 95-090-2]

Monsanto Company; Availability of Determination of Nonregulated Status for Potato Lines Genetically Engineered for Insect 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 that have been genetically engineered for resistance to the Colorado potato beetle 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: May 3, 1996.

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 asked to call in advance of visiting at (202) 690-2817.

FOR FURTHER INFORMATION CONTACT: Dr. James Lackey, Biotechnology Permits, BBEP, APHIS, 4700 River Road Unit 147, Riverdale, MD 20737-1237; (301) 734-7612. To obtain a copy of the determination or the environmental assessment and finding of no significant impact, contact Ms. Kay Peterson at (301) 734-7612; e-mail: mkpeterson@aphis.usda.gov.

SUPPLEMENTARY INFORMATION:

Background

On December 4, 1995, the Animal and Plant Health Inspection Service (APHIS) received a petition (APHIS Petition No. 95-338-01p) from the Monsanto Company (Monsanto) of St. Louis, MO, seeking a determination that two Superior potato lines (SPBT02-5 and SPBT02-7) that have been genetically engineered for resistance to the Colorado potato beetle (CPB) do not present a plant pest risk and, therefore, are not regulated articles under APHIS' regulations in 7 CFR part 340. On December 15, 1995, APHIS received Monsanto's amendment to APHIS Petition No. 95-338-01p to include five additional genetically engineered, CPB-resistant Atlantic potato lines (ATBT04-6, ATBT04-27, ATBT04-30, ATBT04-31, and ATBT04-36).

On January 22, 1996, APHIS published a notice in the Federal Register (61 FR 1557-1558, Docket No. 95-090-1) 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 March 22, 1996. During the designated 60-day comment period, APHIS received three comments on the subject petition, all of which were from potato growers, and all of which were favorable to the petition.

Analysis

Monsanto's two Superior potato lines and five Atlantic potato lines have been genetically engineered to contain the *cryIIIA* gene from the common soil bacterium *Bacillus thuringiensis* subsp. *tenebrionis* (Btt), which encodes a delta-endotoxin insect control protein that is effective against CPB. The subject potato lines also contain the *nptII* gene from the prokaryotic transposon Tn5, which encodes the enzyme neomycin phosphotransferase II and is used as a selectable marker for transformation. Expression of the added genes is controlled in part by 35S promoters from the plant pathogen cauliflower mosaic virus and the 3′ region of the nopaline synthase gene from the plant pathogen *Agrobacterium tumefaciens*. The genes used to develop the subject potato lines were stably transferred into the genome of potato plants through the use of the *A. tumefaciens* transformation system. The parental Superior and Atlantic potato varieties are male fertile, as are the subject potato lines.

The subject Superior and Atlantic potato lines have been considered regulated articles under APHIS' regulations in 7 CFR part 340 because they contain regulatory gene sequences derived from plant pathogens. However, evaluation of field data reports from field tests of the subject potato lines conducted under APHIS permits or notifications since 1992 indicates that there were no deleterious effects on plants, nontarget organisms, or the environment as a result of the release of these potato lines into the environment.

Determination

Based on its analysis of the data submitted by Monsanto and a review of other scientific data, comments received, and field tests of the subject potato lines, APHIS has determined that these potato lines: (1) Exhibit no plant pathogenic properties; (2) are no more likely to become weeds than potatoes 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. Therefore, APHIS has concluded that the subject Superior and Atlantic potato lines and any progeny derived from hybrid crosses with other nontransformed potato varieties will be as safe to grow as potatoes in traditional breeding programs that are not subject to regulation under 7 CFR part 340.

The effect of this determination is that Monsanto's Superior potato lines SPBT02-5 and SPBT02-7 and Atlantic potato lines ATBT04-6, ATBT04-27, ATBT04-30, ATBT04-31, and ATBT04-36 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 (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 Superior potato lines SPBT02-5 and SPBT02-7 and Atlantic potato lines ATBT04-6, ATBT04-27, ATBT04-30, ATBT04-31, and ATBT04-36, 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 9th day of May 1996.

Terry L. Medley,

Acting Administrator, Animal and Plant Health Inspection Service.

[FR Doc. 96-12332 Filed 5-15-96; 8:45 am]

BILLING CODE 3410-34-P

USDA/APHIS Petition 95-338-01p for Determination of Nonregulated Status for Colorado Potato Beetle-Resistant Potato Lines SPBT02-5, SPBT02-7, ABTB04-6, ABTB04-27, ABTB04-30, ABTB04-31, ABTB04-36

Environmental Assessment and
Finding of No Significant Impact

May 1996

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-resistant cv. Superior potato lines SPBT02-5, SPBT02-7 and cv. Atlantic potato lines ABTB04-6, ABTB04-27, ABTB04-30, ABTB04-31, ABTB04-36. 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. This petition is similar to a previous petition, 94-257-01p, from the Monsanto Company. Both are for potato plants with the *cryIIIA* gene. The current petition differs from the previous petition by the recipient potato cultivars (Superior and Atlantic instead of Russet Burbank), and the substitution of a promoter in one of the plasmid constructs. 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 Colorado potato beetle-resistant cv. Superior potato lines SPBT02-5, SPBT02-7 and cv. Atlantic potato lines ABTB04-6, ABTB04-27, ABTB04-30, ABTB04-31, ABTB04-36 shall no longer be regulated articles.

for Michael A. Lidsky (signature) — MAY 03 1996

John H. Payne, Ph.D.
Acting Director
Biotechnology, Biologics, and Environmental Protection
Animal and Plant Health Inspection Service
U.S. Department of Agriculture

Date

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 genetically engineered Colorado potato beetle-resistant cv. Superior potato lines SPBT02-5, SPBT02-7 and cv. Atlantic potato lines ABTB04-6, ABTB04-27, ABTB04-30, ABTB04-31, ABTB04-36, hereafter designated as CPB-resistant potatoes. The Monsanto Company (hereafter referred to as Monsanto), the developer of these CPB-resistant potatoes, petitioned APHIS requesting a determination on the regulated status of these CPB-resistant potatoes. They have been regulated articles under APHIS regulations. This petition is similar to a previous petition, 94-257-01p, from the Monsanto Company. The current petition differs from the previous petition by the recipient potato cultivars (Superior and Atlantic instead of Russet Burbank), and the substitution of a promoter in one of the plasmid constructs. Monsanto has petitioned APHIS for a determination that CPB-resistant 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-resistant potatoes have been developed as an alternative means of providing season-long control of the most damaging pest of potato crops, CPB (Colorado potato beetle, *Leptinotarsa decemlineata* (Say)), on potatoes. The gene conferring resistance to CPB was 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, and a selectable marker gene (*nptII*) encoding the enzyme neomycin phosphotransferase. 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. The genes were introduced via a well-characterized procedure that results in direct introduction of genes into plant genomes.

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

1. CPB-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-resistant potatoes are no more likely to become a weed than insect-resistant potatoes developed by traditional breeding techniques. Potato is not a serious, principal or common weed pest in the U.S., and there is no reason to believe that resistance to CPB would enable potatoes to become weed pests.

3. Multiple barriers insure that gene introgression from CPB-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 impact on biodiversity.

4. Tubers of CPB-resistant potatoes are substantially equivalent in nutritional constituents to nontransgenic tubers and should have no adverse impacts on raw or processed agricultural commodities.

5. CPB-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, or to harm threatened and endangered species.

Therefore, after a review of the available evidence, APHIS believes that CPB-resistant potatoes will be just as safe as nontransgenic potatoes that are typically grown using other methods to control the CPB, 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-resistant potatoes were no longer considered regulated articles under regulations at 7 CFR Part 340.

II. BACKGROUND

A. Development of CPB-Resistant Potatoes.

Monsanto has submitted a "Petition for Determination of Non-regulated Status" to the USDA, APHIS for seven cv. Superior and cv. Atlantic potato lines that are genetically engineered to be resistant to the CPB. Monsanto requested a determination from APHIS that the CPB-resistant potatoes should no longer be considered regulated articles under 7 CFR Part 340.

The gene conferring CPB resistance, originally isolated from the soil bacterium *Bacillus thuringiensis* subsp. *tenebrionis* (*Btt*) Strain BI 256-82, encodes a crystalline protein (delta-endotoxin) designated CryIIIA Btt band three protein, hereafter referred to as CryIIIA protein. Commercial microbial formulations of *Btt* insecticides containing this same protein are registered as pesticides with the Environmental Protection Agency (EPA) for the control of CPB. 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. This region is fused to one of two promoters: The first, in construct PV-STB02, is a promoter derived from the 35S gene of cauliflower mosaic virus (CaMV) with a duplicated enhancer region; or the second, in construct PV-STBT04, is a the small subunit *ats*1A promoter of *Arabidopsis thaliana* ribulose-1,5-bisphosphate carboxylase. The promoter and *cryIIIA* are fused to the 3' nontranslated termination region of a pea ribulose-1,5-bisphosphate carboxylase, small subunit (*rbcS*) gene. The potato lines genetically engineered with this gene construct express the *CryIIIA* protein in their foliage at levels effective at controlling CPB throughout the growing season.

CPB-resistant potatoes have also been transformed with the *nptII* gene from *E. coli* that encodes the enzyme neomycin phosphotransferase II and serves as a selectable marker enabling identification and selection of the transformed plant cells during tissue culture. This gene is fused to the CaMV 35S promoter and the 3' nontranslated termination region of the nopaline synthase gene from *Agrobacterium tumefaciens*, a known plant pest.

These two genes were introduced into CPB-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.

Since 1992, CPB-resistant potatoes have been field tested in the major potato growing regions of the United States under APHIS permits issued (USDA No. 91-360-01r, 92-002-01r, 92-262-02r, 92-363-05r, 93-004-01r) and notifications acknowledged (USDA No. 93-253-06n, 93-357-01n, 93-357-02n, 94-056-01n, 94-056-02n, 94-067-09n, 94-067-10n, 94-074-05n, 94-084-15n, and 94-249-03n). The subject lines of CPB-resistant potatoes have been evaluated extensively in laboratory and field experiments 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 permit 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, (7 U.S.C. 150aa-150jj) as amended, and the Plant Quarantine Act, (7 U.S.C. 151-164a, 166-167) as amended, 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-resistant potatoes described in the Monsanto petition have been considered regulated articles because they contain noncoding DNA regulatory sequences derived from plant pathogens, and because portions of the plasmid vector are 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) (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. A Monsanto application for these potatoes (EPA number 524-UTU) was approved by EPA on May 5, 1995.

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. Residue tolerances for pesticides are established by EPA under the FFDCA; and the FDA enforces the tolerances set by the EPA. A Monsanto request for such an exemption for the plant pesticide active ingredient *Btt* CPB control protein as expressed in these potatoes was granted on April 25, 1995.

The EPA announced a final rule establishing an exemption from the requirement of a tolerance for residues of NPTII and the genetic material necessary for its production when used as a plant pesticide inert ingredient (September 28, 1994, 59 FR 49351-49353, OPP 300362, as it is considered in the CPB-resistant potatoes.

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. 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. The FDA has stated that Monsanto has satisfactorily completed a voluntary food safety consultation with them consistent with this FDA policy statement (Fields, 1994).

III. PURPOSE AND NEED

APHIS has prepared this EA before making a determination on the status of CPB-resistant potatoes as regulated articles under APHIS regulations. The developer of CPB-resistant potatoes, Monsanto, submitted a petition to USDA, APHIS requesting that APHIS make a determination that CPB-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 of 1969 (NEPA) (42 USC 4321 *et seq.*) and the pursuant implementing regulations published by the Council on Environmental Quality (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-resistant potatoes are not regulated articles under the regulations at 7 CFR Part 340. Permits issued or notifications acknowledged by APHIS would still be required for introductions of CPB-resistant potatoes. APHIS might choose this alternative if there were insufficient evidence to demonstrate the lack of plant pest risk from uncontained cultivation of CPB-resistant potatoes.

B. Determination that CPB-Resistant Potatoes Are No Longer Regulated Articles.

Under this alternative, CPB-resistant potatoes would no longer be regulated articles under the regulations at 7 CFR Part 340. Permits issued or notifications acknowledged by APHIS would no longer be required for introductions of CPB-resistant potatoes. A basis for this determination would include a "Finding of No Significant Impact" under the National Environmental Policy Act of 1969 (42 USC 4331 *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 impacts from a determination that CPB-resistant potatoes should no longer be considered regulated articles under APHIS regulations at 7 CFR Part 340. Previous EAs prepared by APHIS prior to the issuance of permits for field tests (see Section II.A. for permit numbers) of CPB-resistant potatoes have addressed various attributes of CPB-resistant potatoes. This EA discusses the genetic modification, and the potential environmental impacts that might be associated with the unconfined cultivation of CPB-resistant potatoes.

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

A. Potential for CPB-Resistant Potatoes to Exhibit Increased Weediness Relative to Traditional Potatoes.

APHIS evaluated whether the CPB-resistant potatoes are any more likely than nontransgenic control 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 defines a plant as a weed if in any specified geographical area, its populations grow entirely or predominantly in situations markedly disturbed by man (without, of course, being deliberately cultivated) (Baker, 1965). He also described the ideal characteristics of weeds (Baker, 1965), 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). They are clonally propagated, late maturing, grown as an annual with tubers from the previous crop. In some agricultural settings potato plants can "volunteer" from tubers left unharvested from the previous growing season or some true potato seed, and persist for several years. These volunteers could pose a weed problem for other crops planted in rotation with potatoes; however, these volunteers are generally controlled with herbicides and cultivation. 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, 1989).

It is unlikely that expression of the *cryIIIA* gene in the CPB-resistant potatoes will provide a competitive advantage sufficient to cause these to become more "weedy" than nontransformed potatoes. Resistance to CPB does not seem to be a critical factor determining weediness in Solanaceous species). None of the characteristics of weeds described by Baker involved resistance or susceptibility to insects. Some *Solanum* species listed as common weeds in the U.S. are not resistant to the CPB, and are common hosts of CPB, but they do have many of the other "weedy" characteristics described by Baker (Correll D.S., 1962; Muenscher, 1955). No cultivated potato varieties are available that are naturally resistant to CPB, but there are many related *Solanum* species in the same subgenus (*Potatoe*), and section (*Petota*) as cultivated potato that are reputed to have resistance to CPB (Germplasm Resources Information Data Base [GRIN], 1994) and that were collected in countries (including the United States) where CPB is listed as a pest (C.A.B. International, 1991). None of these species are listed as serious, principal or common weeds in these countries (Holm et al., 1991).

More importantly, Monsanto presented field data indicating that CPB-resistant potatoes are no more likely than nontransgenic control potatoes to present a plant pest risk as a weed. Control and CPB-resistant potato plants were routinely compared 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 CPB).

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

B. Potential Impacts Associated with Potential Gene Introgression from CPB-Resistant Potatoes to Sexually Compatible Plants (Including Cultivated and Wild Relatives).

APHIS evaluated the potential for gene flow from CPB-resistant potatoes to other cultivated and wild relatives and the potential impacts that this might have on weediness potential of progeny and genetic diversity. The kanamycin resistance trait used as a selectable marker in the CPB-resistant potatoes was not considered in this analysis, because there is unlikely to be selection pressure for this trait in plants in nature.

1) Potential for gene introgression into other potato cultivars and associated potential impacts.

All cultivated potatoes in the U.S. belong to the species *Solanum tuberosum*. Many barriers exist for gene transfer from CPB-resistant potatoes to other potato cultivars or free-living relatives. Barriers to gene introgression into cultivated potatoes include the following: (1) the low acreage planted in male-fertile cultivars reduces the availability of pollen; (2) pollinators (primarily bumblebees) are not attracted to many cultivated potato varieties due to male-sterile flowers and lack of nectar (Helgeson and Davies, 1991; and Plaisted, 1980); and (3) cross-pollination under field conditions is low and is limited by low pollen dispersal rates (Plaisted, 1980; Tynan et al.1990, McPartlan and Dale, 1994).

Therefore, CPB-resistant potatoes are unlikely to outcross to other potato cultivars, and the chances for successful cross-pollination of CPB-resistant potatoes by male-fertile potato cultivars and subsequent seed production will be very unlikely. Introgression into another cultivar would be unlikely to impact genetic diversity of cultivated potatoes in the U.S., because these are vegetatively propagated mostly from certified seed potatoes that are grown under conditions to insure genetic purity. Any transgenic seedlings would be unlikely to persist in the environment because of cultivation or herbicide usage in rotation crops during normal production practices. Transgenic seedlings would be unlikely to have more of a "weediness" potential than volunteer CPB-resistant potatoes, as discussed above.

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

In the unlikely event that male-fertile progeny were produced from CPB-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 *Solanum 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. These barriers include incompatible (unequal) endosperm balance numbers (EBN) that lead to endosperm failure and embryo abortion, multiple ploidy levels, and incompatibility mechanisms that prevent normal pollen tube development and fertilization when two species do not express reciprocal genes to allow fertilization to proceed. No natural hybrids have been observed between these species and cultivated potatoes in the U.S. These barriers exclude the successful introgression of genes from CPB-resistant potatoes into free-living tuber-bearing *Solanum* species occurring in the U.S. Therefore, CPB-resistant potatoes will not impact the genetic diversity of these species. Some accessions of *S. jamesii* and *S. fendleri* from the United States are reputed to already have some resistance to the CPB (GRIN Database, 1994), but neither of these species is listed as a serious, principal or common weed in the United States (Holm et al.,1991; Hanneman, 1994). CPB resistance does not seem to be associated with increased weediness in these species. Therefore, even if the genes for CPB resistance were capable of introgression from the CPB-resistant potatoes into these wild species, this trait would be unlikely to provide a selective advantage sufficient to enable these hybrids to become serious weeds.

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 and our review only extend to the borders of the United States and its territories and possessions. It should be noted however, that all the considerable existing national and international regulatory authorities and phytosanitary regimes that currently apply to introductions of new potato varieties internationally apply equally to the transgenic potatoes covered by this analysis. APHIS is in frequent contact with agricultural officials from many nations, including those with interest in genetically engineered potatoes, to help them develop national scientific and regulatory frameworks that will enable them to make their own scientifically credible decisions about the safety of new crop varieties. Questions have previously been raised 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-resistant potatoes are likely only to be cultivated where CPB is a serious pest and in environments suitable to these cultivars. Of those areas where CPB is currently distributed (C.A.B. International, 1991), Costa Rica, Guatemala and Mexico also contain many wild relatives of cultivated potatoes, and central Mexico is listed as one of the centers of diversity for potatoes (Hawkes, 1990). 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 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. 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.

Introgression in all of these cases would be further limited by barriers previously described. Furthermore, host and habitat preferences of CPB populations in Mexico are such that CPB-resistance is unlikely to provide a selective advantage to many of the wild *Solanum* species, *S. tuberosum* ssp. *andigena* cultivars, or commercial potato cultivars grown there. Therefore, APHIS concludes that the possibility for introgression of Monsanto CPB-resistant potato germplasm into the wild and local cultivars of *Solanum* species in the Central American center of potato diversity is very remote, and the impact (if any) would be minimal. The impact of cultivation of CPB-resistant potatoes on the genetic diversity of wild tuber-bearing *Solanum* populations is likely to be comparable to that from the current cultivation throughout the centers of diversity for potato of traditionally-bred, improved potato varieties.

C. Potential Impacts Associated with Raw or Processed CPB-Resistant Potato Agricultural Products.

APHIS did not evaluate the potential impacts associated with expression of the *Btt* insect control protein and NPTII in raw or processed CPB-resistant potato products and nutritional constituents, because these issues have been addressd by the EPA and FDA as discussed elsewhere in this EA. Because the use of CPB-resistant potatoes may reduce the need to apply insecticides to control CPB, residues of such insecticides may be expected to be lower, on average, in raw or processed agricultural products derived from CPB-resistant potatoes than from those derived from nonmodified potatoes. Likewise, nutritional constituents are being addressed by the FDA based on studies supplied by Monsanto. Information supplied by Monsanto indicate that the transformed potato tubers are similar to nontransformed potatoes in composition.

APHIS has no reason to believe that CPB-resistant potatoes are likely to have any adverse impact on the quality or use of raw or processed agricultural commodities.

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

Consistent with its statutory authority and requirements under NEPA, APHIS evaluated the potential for CPB-resistant potato plants and plant products and the *Btt* insect control protein 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 beneficial organisms, would result from the NPTII protein conferring kanamycin resistance used as a selectable marker during development of Monsanto transgenic potato lines.

1) Potential impact on beneficial and other nontarget arthropods.

APHIS evaluated the results of an extensive field study designed to compare the impact on nontarget arthropods of CPB-resistant potatoes and conventional systemic or foliar insecticides and foliar-applied microbial *Btt* insecticides used to control CPB on nontransgenic. The study was conducted in 1992 at three North American locations (north central Oregon, central Wisconsin, and Prince Edward Island [PEI]) representing different potato production regions with their own respective pest/beneficial insect complexes and appropriate insecticide treatments. The results indicated that CPB-resistant potatoes were more effective than the other CPB control treatments (including foliar-applied microbial *Btt* insecticides) at controlling CPB survival and egg-laying, although all of the treatments provided economically effective levels of protection against defoliation due to CPB. In both Oregon and Wisconsin, generalist predators surveyed during the later part of the season were higher in CPB-resistant potato plots compared to plots conventionally treated for CPB. Significant differences were noted in some species at some locations; in particular more big eyed bug nymphs, damsel bugs, lady bird beetles, hymenopterans (which include parasitic wasps) and spiders were noted. The increased predator populations were sufficient to provide economically acceptable levels of aphid control in CPB-resistant potatoes without supplemental insecticides, whereas the broad spectrum insecticide permethrin, used to control CPB in plots, reduced predator populations significantly and resulted in exponential growth in the aphid population. Although results were inconclusive, CPB-resistant potatoes may also provide some control of another potato pest, the potato flea beetle, which belongs to the same family (Chrysomelidae) as CPB. Two other major pests, potato leafhoppers and wireworms (another coleopteran pest), were not controlled by CPB-resistant potatoes without additional treatments.

A two-year field study at the Oregon site also demonstrated the lack of adverse effects of CPB-resistant potatoes on Collembola (springtails), an order of common beneficial insects that feed on decaying plant material, fungi, and bacteria. The results showed that Collembola populations were higher in CPB-resistant potato plots and plots of potatoes treated with microbial *Btt* insecticide than in plots untreated or treated with conventional systemic insecticide.

Several feeding studies that demonstrate the safety of the *Btt* insect control protein to non-target organisms were submitted by Monsanto to the EPA in support of a Monsanto request for the registration of the *Btt* insect control protein as a plant pesticide and its exemption from the requirement of a tolerance. APHIS considered the results of those high-dose feeding studies that were also submitted by Monsanto in the Petition. Consistent with the results of field studies, no toxic effects on beneficial insects, including adult ladybird beetles, adult parasitic wasps, larvae and adult honeybees, and green lacewing larvae, were reported. Bumblebees and honeybees, as previously discussed, are not attracted to CPB-resistant potatoes because these potatoes lack pollen and nectar.

Monsanto provided further support of the selective toxicity of the *Btt* insect control protein to coleopterans, particularly to CPB. They demonstrated no significant increase in mortality when this protein was fed at a concentration of 50 μg/ml in test diets to nine insect pests of five orders, including two other coleopterans (boll weevil and southern corn rootworm), four lepidopterans (European corn borer, tobacco hornworm, corn earworm, and tobacco budworm) and one dipteran, one orthopteran, and one hemipteran species (yellow fever mosquito, German cockroach, and green peach aphid, respectively). These data support earlier findings by MacIntosh, et al. (1990) who demonstrated no significant insect mortality for these insect species, as well as for three additional coleopteran pests (including white grub, a pest of potato tubers), three additional lepidopteran pests, one isopteran pest and one acarian pest, when fed artificial diets with the *Btt* insect control protein incorporated at a concentration 10-fold higher than that used in the Monsanto study.

2) Potential impact on threatened and endangered arthropods.

The host ranges and habitats of nine coleopteran insect species currently listed or proposed as threatened and endangered in the U.S. were examined to determine if CPB-resistant potatoes might have an adverse impact on these species. None of these species inhabit potato fields or feed on potatoes, and they usually occur in specialized habitats. For example, some of these insects (i.e., the Kretschmarr Cave mold beetle and the Coffin Cave mold beetle) live in caves, and some (i.e., northeastern beach tiger beetle and puritan tiger beetle) live on beaches.

3) Potential impact on other nontarget organisms.

Other invertebrates, such as earthworms, and all vertebrate organisms, including non-target birds, mammals and humans, are not expected to be affected by the *Btt* insect control protein, because they would not be expected to contain the receptor protein found in the midgut of target insects. Results from high dose feeding studies on bobwhite quail, rats and mice demonstrated no adverse effects. Ecological effect studies submitted to the EPA in support of the earlier registration of foliar microbial *Btt* (also called *B.t.* subsp. *san diego*) pesticides indicated no unreasonable adverse effects on nontarget insects, birds, and mammals (EPA, 1988).

APHIS concludes that CPB-resistant potatoes will not have a significant adverse impact on organisms beneficial to plants or agriculture, nontarget organisms, and threatened or endangered species.

E. Potential Impacts on Agricultural Practices Associated with the Cultivation of CPB-Resistant Potatoes.

APHIS considered the potential impacts associated with the cultivation of CPB-resistant potatoes on current agricultural practices used to control CPB.

CPB is the predominant defoliating pest causing costly economic damage to potato crops in the U.S., particularly in the eastern and north central production areas. Both larvae and adult CPB cause severe damage to potato crops. Newly emerged potato plants can suffer severe damage from adults that emerge from overwintering in the soil in nearby fields and crawl (or fly) into newly planted potato fields in the early spring. Cultural control methods, biological and conventional insecticides, and biological control agents currently used or being developed for control of CPB in potatoes are discussed in more detail in the Determination. Foliar microbial *Btt* products have been available since the late 1980's for control of CPB larvae, and no field resistance has been reported, but only approximately 1-2% of acres planted in potatoes nationwide were treated with these products from 1991 to 1993 (New York Agricultural Statistics Service, 1994). Newer *Btt* formulations have improved persistence and efficacy, and as a result they are receiving more wide-spread use, particularly in New York. Conventional chemical insecticides are the primary means of controlling CPB, particularly adult CPBs that appear late in the growing season when other control methods have failed. In 1993, an average of 88% of total acreage planted in fall potatoes in the eleven major states was treated with chemical insecticides (New York Agricultural Statistics Service, 1994). Insecticide resistance is a severe problem in the northeastern potato production region and continues to worsen throughout the north central production region. Because of resistance, many insecticides are becoming obsolete. Two new chemical insecticide formulations of imidacloprid, have recently been registered by the EPA for control of CPB and other pests on potatoes (Rawlings, 1995). There are currently no commercially available potato cultivars that are resistant to CPB. If commercialized, Monsanto transgenic CPB-resistant potatoes could offer an important alternative to chemical insecticides, particularly to those for which resistance has already developed. They will also offer a more flexible, effective alternative for season-long control of CPB compared to the use of some foliar microbial *Btt* products.

Based on this analysis, APHIS concludes that there is unlikely to be any significant adverse impact on agricultural practices associated with the cultivation of CPB-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-resistant potatoes. After careful analysis, APHIS has identified no significant impact to the environment from issuance of a determination that CPB-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-resistant potatoes from its regulation, and has reached the following conclusions:

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

2. CPB-resistant potatoes are no more likely to become a weed than CPB-resistant potatoes which could potentially be developed by traditional breeding techniques. Potato is not a serious, principal or common weed pest in the U.S., and there is no reason to believe that resistance to CPB would lead potatoes expressing this phenotype to become weed pests.

3. Multiple barriers insure that gene introgression from CPB-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 have an adverse impact on biodiversity.

4. Tubers of CPB-resistant potatoes are substantially equivalent in composition to nontransgenic tubers and should have no adverse impacts on raw or processed agricultural commodities.

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

APHIS concludes that CPB-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-resistant potatoes were no longer considered regulated articles under its regulations (7 CFR Part 340).

VII. LITERATURE CITED

ALL LITERATURE CITATIONS CAN BE FOUND IN THE LITERATURE CITATIONS OF THE DETERMINATION.

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RESPONSE TO MONSANTO COMPANY PETITION 95-338-01p FOR DETERMINATION OF NONREGULATED STATUS FOR COLORADO POTATO BEETLE RESISTANT POTATO LINES SPBT02-5, SPBT02-7, ABTB04-6, ABTB04-27, ABTB04-30, ABTB04-31, ABTB04-36

Prepared by
United States Department of Agriculture
Animal and Plant Health Inspection Service
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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 Colorado potato beetle (CPB) (*Leptinotarsa decemlineata*)-resistant cv. Superior potato lines SPBT02-5, SPBT02-7 and cv. Atlantic potato lines ABTB04-6, ABTB04-27, ABTB04-30, ABTB04-31, ABTB04-36 (hereafter referred to as CPB-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-resistant potatoes or their progeny. This petition is similar to a previous petition, 94-257-01p, from the Monsanto Company. The current petition differs from the previous petition by the recipient potato cultivars (Superior and Atlantic instead of Russet Burbank), and the substitution of a promoter in one of the plasmid constructs. This determination by APHIS has been made in response to a petition received from Monsanto Company (Monsanto) on December 4, 1995. The petition requested a determination from APHIS that the CPB-resistant potatoes do not present a plant pest risk and are therefore not regulated articles. On January 22, 1996, APHIS announced receipt of the Monsanto petition in the Federal Register (61 FR 1557-1558) and stated that the petition was available for public view. APHIS invited written comments on this proposed action, to be submitted on or before March 22, 1996.

CPB-resistant potatoes as defined by the developer, Monsanto, were engineered to provide season-long protection from the defoliating pest Colorado potato beetle. To produce the CPB-resistant potatoes, the varieties Superior and Atlantic were genetically engineered by introducing a modified gene, *cryIIIA*, encoding a crystalline delta-endotoxin CryIIIA protein, conferring CPB resistance, originally isolated from the soil bacterium *Bacillus thuringiensis* subsp. *tenebrionis* (*Btt*). Plants were also transformed with a selectable marker gene, *nptII*, encoding neomycin phosphotransferase, originally isolated from *Escherichia coli*. The introduced DNA encoding these genes also has accompanying DNA regulatory sequences that modulate their expression. The DNA regulatory sequences were derived from the plant pathogenic organisms, the bacterium *Agrobacterium tumefaciens* and cauliflower mosaic virus (CaMV) and the nonpathogenic organisms, *Pisum sativum* (pea) and *Arabidopsis thaliana*.

APHIS regulations at 7 CFR Part 340, which were promulgated pursuant to authority granted by the Federal Plant Pest Act (FPPA), (7 U.S.C. 150aa-150jj) as amended, and the Plant Quarantine Act (PQA), (7 U.S.C. 151-164a, 166-167) as amended, 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.

Determination

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-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. In addition, the vector system used to transfer the two genes into the recipient potato was derived from the bacterial plant pathogen, *A. tumefaciens*. Also, certain noncoding regulatory sequences were derived from CaMV and *A. tumefaciens*.

Field testing of CPB-resistant potatoes has been done under APHIS oversight from 1992 to 1995. All field trials were performed under conditions of reproductive confinement.

APHIS has determined that CPB-resistant potatoes do not pose a direct or indirect plant pest risk and therefore will no longer be considered regulated articles under APHIS regulations at 7 CFR Part 340. Oversight under those regulations will no longer be required by APHIS for field testing, importation, or interstate movement of CPB-resistant potatoes or their progeny. (Importation of potatoes derived from CPB-resistant potatoes [and nursery stock or seeds or tubers capable of propagation] is still, however, subject to the restrictions found in the Foreign Quarantine Notice regulations at 7 CFR Part 319.)

This determination is made based on an analysis that revealed that these CPB-resistant potatoes; (1) exhibit no plant pathogenic properties, (2) are no more likely to become a weed than nontransgenic potatoes or CPB-resistant potatoes which could potentially be developed by traditional breeding techniques, (3) are unlikely to increase the weediness potential of any other cultivated plant or native wild species with which the organisms can interbreed, (4) should not cause damage to raw or processed agricultural commodities, and (5) are unlikely to harm other organisms, such as bees, which are beneficial to agriculture or to have adversely impact the ability to control nontarget insect pests, and (6) should pose no greater threat to the ability to control CPB in potatoes and other crops, than that posed by the widely practiced method of applying insecticides to control CPB on potatoes. APHIS has also concluded that there is no reason to believe that new progeny potato varieties derived from CPB-resistant potatoes will exhibit new plant pest properties, i.e., properties substantially different from any observed for the CPB-resistant potatoes already field tested, or those observed for potatoes in traditional breeding programs.

The potential environmental impacts associated with this determination have been examined in accordance with regulations and guidelines implementing the National Environmental Policy Act of 1969 (42 U.S.C. 4331 *et seq.*; 40 CFR 1500-1509; 7 CFR Part 1b). An Environmental Assessment (EA) was prepared and a preliminary Finding of No Significant Impact (FONSI) was reached by APHIS for the determination that CPB-resistant potatoes are no longer regulated articles under its regulations at 7 CFR Part 340. This decision does not release CPB-resistant potatoes from regulations administered by the EPA under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136 *et seq.*) and the Federal Food, Drug, and Cosmetic Act (FFDCA) (21 U.S.C. 301 *et seq.*).

The body of this document consists of three parts: (1) background information that provides the legal framework under which APHIS has regulated the field testing, interstate movement, and importation of CPB-resistant potatoes, (2) a summary of and response to comments provided to APHIS on its proposed action during the public comment period; and (3) analysis of the key factors relevant to the APHIS decision that the CPB-resistant potatoes do not present a plant pest risk.

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), (7 U.S.C. 150aa-150jj) as amended, and the Plant Quarantine Act (PQA), (7 U.S.C. 151-164a, 166-167) as amended, 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.

The CPB-resistant potatoes have been considered "regulated articles" under Part 340 of the regulations in part because the vector system used to transfer the two genes into the potato genome was derived from *A. tumefaciens*, a known plant pathogen. In addition, certain noncoding regulatory sequences were derived from plant pathogens, i.e., from CaMV and *A. tumefaciens*. The decision by APHIS that CPB-resistant potatoes are not regulated articles is based in part on evidence provided by Monsanto concerning the biological properties of CPB-resistant potatoes and their similarity to other varieties of potatoes grown using standard agricultural practices for commercial sale or private use.

A determination that an organism does not present a plant pest risk can be made under this definition, especially when there is evidence that the plant under consideration: 1) exhibits no plant pathogenic properties; 2) is no more likely to become a weed than the non-modified parental variety; 3) is unlikely to increase the weediness potential of any other cultivated plant; 4) does not cause damage to processed agricultural commodities; and 5) is unlikely to harm other organisms that are beneficial to agriculture. Evidence has been presented by Monsanto that bears on these topics. In addition, it should be established that there is no reason to believe that any new potato varieties bred from CPB-resistant potatoes will exhibit plant pest properties substantially different from any observed for potato in traditional breeding programs, or as seen in the development of CPB-resistant potatoes already field tested.

B. EPA and FDA regulatory authority.

The CPB-resistant potato lines are currently subject to regulations administered by the EPA or the FDA (described in Section II.C. of the Environmental Assessment) that require registration of pesticides prior to their distribution and sale and establish tolerances for pesticide residues in raw agricultural products. APHIS decision on the regulatory status of the CPB-resistant potatoes under APHIS regulations at 7 CFR 340, in no way releases these potatoes from EPA and FDA regulatory oversight.

III. RESPONSE TO COMMENTS

APHIS received 3 comments on the subject petition from potato growers. All commenters expressed support for the petition. Two growers provided assurances that the CPB-resistant potato varieties had the same agronomic traits as nontransformed potatoes except for the CPB resistance. All growers stressed the need for the product because of the critical problem of CPB.

IV. ANALYSIS OF THE PROPERTIES OF CPB-RESISTANT POTATOES

A brief discussion of potato biology follows in the next paragraph to help inform the subsequent analysis. This information is expanded in subsequent sections when it is relevant in addressing particular risk assessment issues.

The potato (*Solanum tuberosum* L.) is a major food crop in North and South

America, Europe, and Russia, with an exceptionally high yield per acre (Burton, 1969); and it is used in a wide variety of table, processed, livestock feed, and industrial uses (Feustel, 1987; Talburt, 1987). Potatoes are grown throughout the U.S. where agronomic conditions will permit an economic yield to be obtained, but particularly in the most northern states (excluding Alaska).

Potato belongs to the Solanaceae which have about 90 genera and 2,800 species and which also include tomatoes, peppers, tobacco, and eggplant. The family is found throughout the world, but is especially concentrated in the tropical regions of Latin America (Correll, 1962). The genus *Solanum*, to which potato and all wild relatives belong, consists of about 2,000 species. Within this genus, the section Petota (D'Arcy, 1972), also known as section Tuberarium (Dodds, 1962), includes the tuber-bearing members, of which the cultivated potato is best known. The wild species of the section Petota, numbering about 180, are prominent in the Peruvian and Bolivian Andes; they have been subject to repeated germplasm collecting expeditions, and still represent a rich source of diversity in breeding programs (Correll, 1962; Ross, 1986).

Cultivated potatoes are an herbaceous, clonally propagated crop grown as an annual. The flowers are perfect, but are typically outcrossed, and require insects for pollination, in particular bumblebees. The fruits are berries, but are absent in many cultivars (Burbank, 1921) due to many factors which can block successful fertilization or seed set. Potato plants are noted for their sterility (Ross, 1986), and this causes difficulties in potato breeding. Tubers form underground from rhizomes (Burton, 1969). Commercial potato "seed" is not a true botanical seed, but rather consists of sections of potato tuber with one or more "eyes", i.e. lateral buds (Everett, 1981). The commercial potatoes are therefore all reproduced vegetatively as clones. This means that once a cultivar is produced it is genetically stable in perpetuity, barring mutation, or some other unusual event. It also means that potato clones are especially susceptible to disease transmission via the tuber sections (Ross, 1986). For this reason, many farmers plant only certified seed.

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

The seven CPB-resistant potato lines were produced using an *Agrobacterium*-meditated transformation protocol to transform (by seven independent transformations events) the Superior and Atlantic cultivars with genes encoding the CryIIIA protein, conferring resistance to CPB, and the neomycin phosphotransferase type II protein, conferring resistance to the antibiotic kanamycin. 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 bacteria *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.

The gene encoding the protein neomycin phosphotransferase type II (also called NPTII or aminoglycoside 3'-phosphotransferase II) was isolated from a transposon contained in a strain of *E. coli* K12 (Beck et al., 1982; Jorgensen et al., 1979). *E. coli*, a common enteric bacterium found in the human gut, is not a regulated article. This gene was introduced as a selectable marker, i.e., as a tag enabling identification of potato cells that had concomitantly taken up the *cryIIIA* gene. Following transformation, plant cells expressing the enzyme NPTII can survive laboratory selection on the antibiotic kanamycin because NPTII deactivates, by phosphorylation, aminoglycoside antibiotics such as kanamycin. Its use does not result in the presence of the antibiotic kanamycin in CPB-resistant potatoes, and its presence does not imply that kanamycin will be used in the cultivation of these potatoes.

The introduced DNA that encodes the *cryIIIA* and *nptII* genes also has accompanying DNA regulatory sequences that modulate the expression of these genes in plants. The DNA regulatory sequences were derived from nonpathogenic organisms, *Pisum sativum* (pea) and *Arabidopsis thaliana*, and two organisms which are plant pathogens: the bacterium *A. tumefaciens* and CaMV. Specifically, the DNA regulatory sequences associated with the *cryIIIA* gene comprise one of two promoters: The first, in construct PV-STB02, is a promoter derived from the 35S gene of cauliflower mosaic virus (CaMV) with a duplicated enhancer region (Kay et al., 1987; Odell et al., 1985); or the second, in construct PV-STBT04, is a the small subunit *ats*1A promoter of *Arabidopsis thaliana* ribulose-1,5-bisphosphate carboxylase; and the 3' nontranslated region of the pea ribulose-1,5-bisphosphate carboxylase, small subunit (*rbcS*) E9 gene (Coruzzi et al., 1984) which functions to terminate transcription and direct polyadenylation of the cryIIIA mRNA. The DNA regulatory sequences associated with the *nptII* gene comprise the CaMV 35S promoter (Gardner et al., 1981; Sanders et al., 1987) and the 3' nontranslated region of the nopaline synthase gene from *A. tumefaciens*, which functions to terminate transcription and direct polyadenylation of the *nptII* gene (Depicker et al., 1982; Bevan et al., 1983). Although these regulatory sequences were derived from plant pathogens, the regulatory sequences cannot cause plant disease by themselves or with the genes that they regulate.

CPB-resistant potato plants were derived by transforming Superior and Atlantic varieties via a well-characterized technique that uses DNA sequences from *A. tumefaciens* to introduce those genes subcloned between the T-DNA borders into the chromosome of the recipient plant (Klee and Rogers, 1989; and Zambryski, 1988). Although some DNA sequences used in the transformation process were derived from 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 and sexually-transmitted in the same manner as any other genes.

Analyses of the different CPB-resistant potato lines indicated that one, two, or three copies of the introduced genes were inserted into the chromosomal DNA.

During extensive field testing, the CPB-resistant potatoes exhibited the typical agronomic characteristics of the recipient Superior or Atlantic varieties, with the exception of the desired CPB-resistant phenotype conferred by the *Btt* insect control protein. In APHIS opinion, the components, quality and processing characteristics of CPB-resistant potatoes reveal no differences that could have an indirect plant pest effect on any raw or processed plant commodity. CPB-resistant potatoes exhibit no plant pest characteristics.

B. CPB-resistant potatoes have no significant potential to become successful weeds.

APHIS evaluated whether the CPB-resistant potatoes are any more likely than nontransgenic control potatoes to present a plant pest risk as a weed. Most definitions of weediness stress as core attributes the undesirable nature of weeds from the point of view of humans; from this core, individual definitions differ in approach and emphasis (Baker, 1965; de Wet and Harlan, 1975; Muenscher, 1955). Baker defines a plant as a weed if, in any specified geographical area, its populations grow entirely or predominantly in situations markedly disturbed by man (without, of course, being deliberately cultivated) (Baker, 1965). He also described the ideal characteristics of weeds as including the following: discontinuous germination and long-lived seeds; rapid seedling growth; rapid growth to reproductive stage; long continuous seed production; self-compatible, but not obligatorily self-pollinated or apomictic; if outcrossing, uses wind or unspecialized pollinator; high seed output under favorable conditions; germination and seed production under a wide range of environmental conditions; high tolerance or plasticity of climatic and edaphic variation; special adaptations for dispersal; good competitiveness achieved through, for example, allelochemicals or choking growth; and if perennial, then with vigorous vegetative reproduction, brittleness at the lower nodes or of rhizomes or rootstocks, and ability to regenerate from severed rootstocks. Although Baker's characteristics have been criticized, no more broadly accepted suite of characteristics has 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 Baker's "weedy" characteristics (Keeler, 1989). It is a clonally propagated, late maturing plant, grown as an annual with tubers from the crop of the previous year serving as propagules. In agricultural settings where mild winter conditions or heavy snow cover exist, potato plants can "volunteer" from tubers left unharvested from the previous growing season, and persist for several years. These volunteers could pose a weed problem for other crops planted in rotation with potatoes; however, these volunteers are generally controlled with herbicides and cultivation. 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 in most weed compendia (Holm et al., 1991; Muenscher, 1955; USDA, 1971; Weed Science Society of America, 1989).

It is unlikely that expression of the CPB insect control protein in the CPB-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 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; e.g. 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 *Potatoe*, 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). The susceptibility of potatoes to many potato diseases (most virus diseases, late blight, and *Fusarium* and *Verticillium* wilts) (Thompson, 1987) will also limit their competitiveness or persistence as a weed.

Monsanto provided data from field experiments conducted in three geographically diverse potato production areas (Oregon, Wisconsin, and New York) during the winter of 1993-1994 to determine overwintering survival of CPB-resistant potatoes compared to control potatoes. Their data indicate that CPB-resistant potatoes do not have an increased ability to become weeds by overwintering in cultivated potato- producing areas. In Wisconsin and New York, where subzero temperatures were reached and snow fall was minimal, no volunteers were observed. In Oregon, where temperatures were milder, 65-70% of the tubers of both controls and CPB-resistant potatoes sprouted volunteers the following spring. Percent stand (emergence) and yield of potatoes in field trials in Idaho and Washington were evaluated, as these may be indicators of the fitness and number of potential propagules available to volunteer. No significant differences were observed between CPB-resistant potatoes and the nontransgenic potatoes.

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

C. Multiple barriers insure that gene introgression from CPB-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-resistant potatoes to other cultivated and wild relatives. Then two potential impacts that might result from this sexual transfer of genes were evaluated: first, that the traits from CPB-resistant potatoes might cause free-living relatives to become "weedier", and second, that the transfer of genes might cause population changes that would lead to reduced genetic diversity. The kanamycin resistance trait used as a selectable marker in the CPB-resistant potatoes was not considered in this analysis, because there is no selection pressure for this trait in plants in nature (i.e., kanamycin is not applied to field crops).

1) Potential for gene introgression into other potato cultivars and associated potential impacts.

Many barriers exist to impede gene transfer from CPB-resistant potatoes to other potato cultivars or free-living relatives. All cultivated potatoes in the U.S. belong to the species *Solanum tuberosum*. The only pathway for sexual gene flow would be for the CPB-resistant potatoes to cross with a sexually-compatible species. The probability of this is low due to the relatively low acreage planted in male-fertile cultivars. Many of the most popular cultivated potato varieties grown in the U.S. and Canada have little or no pollen (National Potato Council, 1994). Self-pollination is more prevalent than cross-pollination, because pollinators (primarily bumblebees) are not attracted to most cultivated potato varieties due to male-sterile flowers and lack of nectar (Helgeson and Davies, 1991; Plaisted, 1980). Estimates of the range of cross-pollination under field conditions range from 0 to ca. 20% (Plaisted, 1980). Many studies using male-fertile transgenic plants have been conducted to examine pollen movement in potatoes. In New Zealand, Tynan et al.(1990) showed that with the cultivar "CRD Iwa", the percentage of transgenic progeny obtained from non-transgenic plants ranged from 1% when the plants were interplanted to none at all when nontransgenic and transgenic plants were separated by more than 4.5 meters. In Cambridge, UK, McPartlan and Dale (1994) showed that when transgenic and non-transgenic plants of the variety "Desiree" were planted in alternate rows (such that leaves were touching), 24% of seedlings from the non-transgenic parent plants were transgenic. The frequency of transgenic progeny dropped to 2% and 0.017% when the distance was lengthened to 3 and 10 meters, respectively, and no transgenic progeny were obtained when the distance was 20 meters.

Therefore, due to the large number of male-sterile potato plants under production, low pollen dispersal rates of male-fertile potato varieties, and lack of incentive for insect pollination, the chance for successful cross-pollination of CPB-resistant potatoes by male-fertile potato cultivars will be small. Such an occurrence would be unlikely to impact genetic diversity of cultivated potatoes in the U.S. This is because most potato production is initiated from vegetative certified seed potatoes; and in fact, some states require that only certified seed potatoes be planted. Certified seed potatoes are grown under conditions to insure genetic integrity. Any transgenic seedlings would be unlikely to persist in the environment because of cultivation or herbicide usage in rotation crops during normal production practices. In the U.S., potatoes are rotated with other crops on a cycle of two to five years. Transgenic seedlings would be unlikely to have more of a "weediness" potential than volunteer CPB-resistant potatoes, as discussed above.

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

In the unlikely event that male-fertile transgenic progeny are produced from CPB-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. APHIS also evaluated the potential environmental impacts associated with such events.

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.

Other barriers exist that have prevented attempts to hybridize these wild species directly with cultivated *S. tuberosum* under natural field conditions or using natural (non-chemically assisted) hybridization techniques. 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*.

S. tuberosum (4x) has a higher ploidy level than *S. jamesii* and *S.*

pinnatisectum, which are both diploid (2x). Increasing the ploidy level of the wild species through the production of unreduced gametes is one potential natural way of increasing their EBNs to be compatible with that of *S. tuberosum*, but numerous attempts to produce hybrids using this technique have failed. Even if they were to succeed, the progeny (with an EBN of 4) would not be compatible for further hybridization back to the wild species (with lower EBNs), and introgression would cease.

Incompatibility systems prevent normal pollen tube development and fertilization when two species do not express reciprocal genes to allow fertilization to proceed. This type of incompatibility has been observed in crosses between *S. tuberosum* and *S. fenderlii*. Evidence exists that different numbers of genes control the incompatibility systems present in South American potato species (from which *S. tuberosum* was derived) and some Mexican species (including *S. pinnatisectum*), and this could theoretically lead to incompatibility.

These barriers, along with other barriers to introgression from cultivated potatoes, insure that gene introgression from transgenic cultivated potatoes into free-living tuber-bearing *Solanum* species in the U.S is improbable. Professor John Hermsen, Agricultural University, Wageningen, the Netherlands, during the Workshop on Safequards for Planned Introduction of Transgenic Potatoes (1991), presented essentially these same arguments when he also concluded that gene flow from transgenic cultivated *S. tuberosum* into the natural ecosystem in the United States is virtually excluded. Therefore, CPB-resistant potatoes will not impact the genetic diversity of these species. Even in the unlikely event that the gene for CPB resistance were to introgress into these species, this new trait would be unlikely to provide a selective advantage that would enable such hybrids to become serious weeds. The GRIN Database (1994) lists 13 accessions of *S. jamesii* and 1 accession of *S. fendleri* from the United States reputed to already have resistance to the CPB. Despite such observed resistance, neither of these species is listed as a serious, principal or common weed in the United States by Holm et al. (1991) nor are they described as weeds by Hanneman (1994). Therefore, introgression of CPB resistance into these wild species would not be expected to enable these plants to become serious weeds.

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, because our authority and our review only extend to the borders of the United States and its territories and possessions. APHIS is in frequent contact with agricultural officials from many foreign nations, including those with interest in genetically engineered potatoes, to help them develop national scientific and regulatory frameworks that will enable them to make their own scientifically credible decisions about the safety of new crop varieties. Questions have previously been raised 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-resistant potatoes are likely to be cultivated only where CPB is a serious pest and in environments to which it is suited. Therefore, APHIS evaluated the environmental impacts of gene introgression into wild relatives that occur where CPB is a pest. 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.

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-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). This species may have potential value for improving cultivated potato strains because of resistance to blight and frost. *S. demissum* is reported to have poisonous components (glycoalkaloids) in the leaves which 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.

Determination

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-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).

There is already considerable cultivation throughout the centers of diversity for potato of improved potato varieties produced through crop breeding. The impact of cultivation of CPB-resistant potatoes on the genetic diversity of wild tuber-bearing *Solanum* populations is likely to be comparable to that from these other nontransgenic improved varieties.

We note also that any international traffic in CPB-resistant potatoes would be fully subject to national and regional phytosanitary standards promulgated under the International Plant Protection Convention (IPPC). The IPPC has set a standard for the reciprocal acceptance of phytosanitary certification among the nations that have signed or acceded to the Convention (98 countries as of December 1992). The treaty, administered through the United Nations Food and Agriculture Organization, came into force on April 3, 1952. It establishes standards to facilitate the safe movement of plant materials across international boundaries. Plant biotechnology products are fully subject to national legislation and regulations, or regional standards and guidelines promulgated under the IPPC. The vast majority of IPPC signatories have promulgated, and are now administering, such legislation or guidelines. Mexico in particular has in place a regulatory process that would require a full evaluation of the CPB-resistant potatoes before they could be introduced into their environment. Our decision in no way prejudices regulatory action in any country. The IPPC has also led to the creation of regional plant protection organizations such as the North American Plant Protection Organization (NAPPO) whose member countries are the U.S., Canada, and Mexico. Our trading partners will be kept informed of our regulatory decisions through NAPPO, and other fora. In addition to the assurance provided by the analysis leading APHIS to a Finding of No Significant Impact for the introduction of this potato variety, it should be noted that all the considerable existing national and international regulatory authorities and phytosanitary regimes that currently apply to introductions of new potato varieties internationally apply equally to the transgenic potatoes covered by this analysis.

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

agricultural commodities.

APHIS did not evaluate the potential impacts associated with expression of the *Btt* insect control protein and NPTII in raw or processed CPB-resistant potato products, because these issues are addressed by the EPA and FDA.

The EPA is responsible for the regulation of pesticides under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (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. A Monsanto application for these potatoes (EPA number 524-UTU) was approved by EPA on May 5, 1995.

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. Residue tolerances for pesticides are established by EPA under the FFDCA; and the FDA enforces the tolerances set by the EPA. A Monsanto request for such an exemption for the plant pesticide active ingredient *Btt* CPB control protein as expressed in these potatoes was granted on April 25, 1995.

The EPA announced a final rule establishing an exemption from the requirement of a tolerance for residues of NPTII and the genetic material necessary for its production when used as a plant pesticide inert ingredient (59 FR 49351-49353, Docket No. 94-23762), as it is considered in the CPB-resistant potatoes.

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. 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.

Because the use of CPB-resistant potatoes may reduce the need to apply insecticides to control CPB, residues of such insecticides might be expected to be lower, on average, in raw or processed agricultural products derived from CPB-resistant potatoes than from those derived from nonmodified potatoes.

Based on these analyses, APHIS has no reason to believe that CPB-resistant potatoes are likely to have any adverse impact on the quality or use of raw or processed agricultural commodities.

E. CPB-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 CPB-resistant potato plants and plant products and the *Btt* insect control protein to have damaging or toxic effects directly or indirectly on nontarget organisms, particularly those that are recognized as beneficial to agriculture. APHIS also considered potential impacts on other nontarget pests, because such impacts could have an impact on the potential for changes in agricultural practices. There is no reason to believe that the NPTII protein conferring kanamycin resistance in the CPB-resistant potato plants as a selectable marker for transformation would have deleterious effects or significant impacts on nontarget organisms, including beneficial organisms. There have been no reports of toxic effects on such organisms in the many field trials conducted with many different plants expressing this selectable marker.

1) Potential impact on beneficial arthropods and other nontarget arthropods and pests.

APHIS evaluated the results of field studies and toxicity studies submitted by Monsanto as part of the petition. Data were submitted from an extensive study that evaluated the impacts on nontarget arthropods of CPB-resistant potatoes compared to conventional systemic or foliar insecticides and foliar-applied microbial *Btt* insecticides used to control CPB on non-transgenic. The study was conducted in 1992 at three North American locations (north central Oregon, central Wisconsin, and Prince Edward Island [PEI]) representing different potato production regions with their own respective pest/beneficial insect complexes.

In Oregon, CPB-resistant potatoes with no treatment or with systemic insecticides for aphid control were compared to untreated or treated with either a foliar, broad spectrum insecticide (permethrin) or a microbial *Btt* formulation for CPB control, or with a systemic insecticide for CPB and aphid control. In Wisconsin, CPB-resistant potatoes treated with a foliar insecticide for selective potato leafhopper control, were compared to potatoes treated in one of the following ways: 1) with conventional foliar insecticides for CPB, potato leafhopper and aphid control, 2) with foliar-applied microbial *Btt* and supplemental insecticides for adult CPB and selective potato leafhopper control, or 3) untreated for CPB, but selectively treated for potato leafhopper. In PEI, the insect pressure was low due to a cold winter and only the following different treatments were compared: CPB-resistant potatoes both untreated or treated with systemic insecticide for control of CPB, potato flea beetle and aphids. The treatments were replicated four to six times, depending on the site. Pests (and their damage) and beneficial predatory or parasitic insects were assayed throughout the growing season using sampling techniques effective for both flying and sedentary foliage-dwelling insects, and crawling ground insects.

The results indicated that CPB-resistant potatoes were more effective than the other CPB control treatments (including foliar-applied microbial *Btt*) at controlling CPB survival and egg-laying, although all of the treatments for controlling CPB at all of the locations provided economically effective levels of protection against defoliation due to CPB. Therefore, the relative effects on nontarget insects are meaningful on a practical level. In both Oregon and Wisconsin, beneficial generalist predators surveyed during the later part of the season were higher in plots of CPB-resistant potatoes compared to plots of potatoes conventionally treated for CPB. In Oregon, 97% of predators were composed of big eyed bugs (family: Geocoridae), damsel bugs (family: Nabidae), minute pirate bugs (family: Anthocoridae), and spiders. Other observed predators included lady beetles (family: Coccinellidae), brown lacewings (family: Hererobiidae), flower flies (family: Syrphidae) and stink bugs (family: Pentatomidae). CPB-resistant potatoes treated with microbial foliar-applied *Btt* both had significantly more big eyed bug nymphs, damsel bugs and spiders than the other treatments sampled in mid- to late season. Vacuum sampling of the Wisconsin plots during the late season showed that, compared to conventionally-treated potato plots, CPB-resistant potato plots had significantly greater populations of anthocorids, coccinellids, and hymenopterans (which include parasitic wasps), and did not have significantly less spiders or beneficial predators from any of the other families surveyed including Chrysopidae (common lacewings), Carabidae (ground beetles), and Reduviidae (assassin bugs, ambush bugs and thread-legged bugs). PEI predator populations were low due to the low food source (pests), and no significant differences were observed between plots.

The increased predator populations were sufficient to provide economically acceptable levels of aphid control in CPB-resistant potatoes without supplemental insecticides, whereas the broad-spectrum insecticide permethrin, used to control CPB in potatoes, reduced predator populations 5-8 fold, and resulted in exponential growth in the aphid population. CPB-resistant potatoes may also have an impact on another potato pest, the potato flea beetle (*Epitrix cucumeris*), which belongs to the same family (Chrysomelidae) as CPB. In Wisconsin, significantly fewer potato flea beetles were recovered from CPB-resistant potato plots compared to foliar-applied microbial *Btt*-treated plots. Even though adult potato flea beetle populations were not reduced, significantly reduced feeding damage compared to untreated plots was observed on PEI. Two other major pests, potato leafhopper and wireworm (another coleopteran pest), were not controlled by CPB-resistant potatoes without additional treatments.

A two-year field study at the Oregon site also demonstrated the lack of adverse effects of CPB-resistant potatoes on Collembola (springtails), an order of common beneficial insects that feed on decaying plant material, fungi, and bacteria. The results showed that Collembola populations were higher in plots containing untreated CPB-resistant potatoes or potatoes treated with microbial *Btt* pesticide than in plots untreated or treated with conventional systemic insecticide.

Before a product may be registered by the EPA as a pesticide under FIFRA, it must be shown that when used in accordance with widespread and commonly recognized practice, it will not generally cause unreasonable adverse effects on the environment. Several feeding studies demonstrating the safety of the *Btt* insect control protein to nontarget organisms have been submitted by Monsanto to the EPA in support of Monsanto request for the registration of the *Btt* insect control protein as a plant pesticide and its exemption from the requirement of a tolerance. APHIS evaluated the results of those feeding studies that were also submitted by Monsanto in the petition. Consistent with the results of field studies, no toxic effects on beneficial insects, including adult ladybird beetles (*Hippodamia convergens*), adult parasitic wasps (*Nasonia vitripennis*), larvae and adult honeybees (*Apis mellifera*), and green lacewing larvae (*Chrysopa carnea*), were reported. Insects that were fed *Btt* insect control protein in their diet for up to 10 days at concentrations at least 100 times higher than the LC_{50} (1ppm) for CPB, exhibited a similar percent mortality to those fed control diets without the insecticidal protein. These concentrations are very high compared to the mean expression level of *Btt* insect control protein in CPB-resistant potato young leaf tissue (19.1 μg/g fresh weight or 19.1 ppm) and tubers (1.01 μg/g fresh weight or 1.01 ppm). Bumblebees and honeybees would also be unlikely to be impacted by the CPB-resistant potatoes, because as discussed above, they are not attracted to these potatoes because these potatoes lack nectaries and pollen.

In further support of the selective toxicity of the *Btt* insect control protein to coleopteran insects, particularly to CPB, Monsanto demonstrated no significant increase in mortality when this protein was fed at a concentration of 50 μg/ml in test diets to nine other insect pest species from five orders, i.e., two other coleopterans, *Anthomonis grandis* (boll weevil) and *Diabrotica undecimpunctata* (southern corn rootworm); four lepidopterans, *Ostrinia nubialis* (European corn borer), *Manduca sexta* (tobacco hornworm), *Helicoverpa zea* (corn earworm), *Heliothis virescens* (tobacco budworm); one dipteran, *Aedes aegypti* (yellow fever mosquito); one orthopteran *Blatella germanica* (German cockroach); and one hemipteran, *Myzus persicae* (green peach aphid). In the green peach aphid there was a very slight reduction in honeydew production, indicating reduced feeding. These data support earlier findings by MacIntosh, et al. (1990) who demonstrated no significant insect mortality from the *Btt* insect control protein for the insect species listed above, as well as for eight additional agronomically important insect pests: i.e., three additional coleopterans (including white grub, a pest of potato tubers), three additional lepidopterans, one isopteran and one acarian. In this study, insects were fed artificial diets with the *Btt* insect control protein incorporated at a concentration 10-fold higher than that used in Monsanto study, and almost 100-fold higher than the LC_{50} of 6.5 μg/ml for CPB.

2) Potential impacts on other nontarget organisms

Other invertebrates, such as earthworms, and all vertebrate organisms including nontarget birds, mammals and humans, are not expected to be affected by the *Btt* insect control protein, because they would not be expected to contain the receptor protein found in the midgut of target insects.

APHIS concludes that CPB-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-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-resistant potatoes or their progeny. (Importation of CPB-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:

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

2. CPB-resistant potatoes are no more likely to become a weed than CPB-resistant potatoes which could potentially be developed by traditional breeding techniques. Potato is not a serious, principal or common weed pest in the U.S., and there is no reason to believe that resistance to CPB would enable potatoes to become weed pests.

3. Multiple barriers insure that gene introgression from CPB-resistant potatoes into wild or cultivated sexually-compatible plants is extremely unlikely, and such rare events should not increase the weediness potential of any resulting progeny or adversely impact biodiversity.

4. Tubers of CPB-resistant potatoes are substantially equivalent in composition, quality and characteristics to nontransgenic tubers and should have no adverse impacts on raw or processed agricultural commodities.

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

APHIS has also concluded that if any new varieties are bred from CPB-resistant potatoes they are unlikely to exhibit new plant pest properties, i.e., properties substantially different from any observed for CPB-resistant potatoes already field tested, or those observed for potatoes developed in traditional breeding programs.

Michael A. Lidsky (for) — MAY 03 1996

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U.S. Department of Agriculture

VI. LITERATURE CITED

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