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 Russet Burbank potato lines BT6, BT10, BT12, BT16, BT17, BT18, AND BT23, 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. Interstate movements and field tests of CPB-resistant potatoes have been conducted under permits issued or through notifications acknowledged by APHIS. 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 Russet Burbank potatoes, currently the most widely grown potato cultivar in the United States. The gene conferring resistance to CPB was introduced via genetic engineering techniques. These techniques enabled the developer to express in Russet Burbank potato plants a gene 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 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 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, quality and French fry characteristics to nontransgenic Russet Burbank 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.

6. Development of resistance to insecticides is a potential risk associated with their use; but in this respect, cultivation of CPB-resistant potatoes should pose no greater threat to the control of CPB in potatoes and other crops, than that posed by the widely practiced method of applying insecticides to control CPB on potatoes.

Therefore, after a review of the available evidence, APHIS believes that CPB-resistant potatoes will be just as safe as nontransgenic Russet Burbank 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 Russet Burbank 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*), encodes a crystalline protein (delta-endotoxin) designated CryIIIA Btt band three protein. Commercial microbial formulations of *Btt* insecticides containing this same protein are registered as pesticides with the Environmental Protection Agency 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 protein coding region of the gene was modified with plant preferred amino acid codons for optimal expression in plants. This region is fused to the promoter derived from the 35S gene of cauliflower mosaic virus (CaMV) with a duplicated enhancer region and to the 3' nontranslated termination region of a pea ribulose-1,5-bisphosphate carboxylase, small subunit (*rbcS*) gene. The Russet Burbank potato lines

genetically engineered with this gene construct express the *Btt* insecticidal 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 1991, CPB-resistant potatoes have been (and/or are currently being) field tested in the major potato growing regions of the United States under APHIS permits issued (USDA No. 91-011-04r, 91-050-02r, 91-360-01r, 92-002-01r, 92-002-02r, 92-262-02r, 92-363-05r, 93-004-01r) and notifications acknowledged (USDA No. 94-007-03n, 94-056-01n, 94-056-02n, 93-056-03n, 94-067-09n, 94-067-10n, 94-080-06n, 94-084-15n, 94-089-02n, 94-249-03n, 94-357-01n, and 94-357-02n). 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. Accordingly, Monsanto has voluntarily submitted to EPA an application to register this plant-pesticide, ie. the *Btt* CPB control protein as produced by the *cry IIIA* gene and its controlling sequences in these genetically engineered potato lines. On December 8, 1993, EPA announced receipt of this application (EPA File Symbol 524-UTU) in the Federal Register (58 FR 64582-64583). This is the first application for registration of a transgenic plant pesticide under section 3(c) of FIFRA, as amended, in which a plant has been genetically altered to produce a pesticide. The EPA has not yet announced its final decision on this registration application; however, the Office of Pesticides Program (OPP) has made available in the public docket a preliminary scientific position document regarding this registration application in preparation for a FIFRA Scientific Advisory Panel meeting, announced in the Federal Register, January 25, 1995, Docket No. 95-2009, p. 4910. Before a product may be registered 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. FIFRA also authorizes EPA to issue Experimental Use Permits (EUP) and otherwise regulate the use of unregistered pesticides under FIFRA section 3(a). EUPs are generally issued (as authorized under FIFRA section 5 and 40 CFR part 172) for large-scale testing of pesticides.

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. Monsanto has submitted to the EPA a pesticide petition (PP 3F4273) proposing to amend 40 CFR part 180 to establish a tolerance exemption for residues of the plant pesticide active ingredient *Btt* CPB control protein as expressed in plant cells. On December 8, 1993, EPA announced receipt of this petition [58 FR 64583-64584]. The EPA has not yet announced its decision on this petition. The EPA has already 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. FDA's 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 (NEPA) of 1969 (40 CFR 1500-1508) and the pursuant implementing regulations published by the Council on Environmental Quality (42 USC 4331 et seq.; 40 CFR 1500-1508; 7 CFR Part 1b; 44 FR 50381-50384; and 44 FR 51272-51274).

Consistent with the "Coordinated Framework for Regulation of Biotechnology" (51 FR 23302-23350, June 26, 1986), when appropriate, APHIS and the EPA have been coordinating their review of these genetically engineered potato lines to avoid duplication and assure that all relevant issues are addressed. Therefore, reference is made to EPA review documents that address certain environmental issues.

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 1500-1509; 7 CFR Part 1b; 44 FR 50381-50384; and 44 FR 51272-51274).

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 Russet Burbank Potatoes

APHIS evaluated whether the CPB-resistant potatoes are any more likely than nontransgenic control Russet Burbank 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 core, 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 by some ecologists as nonpredictive, no more broadly accepted suite 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, and particularly the Russet Burbank variety, lacks most of these "weedy" characteristics (Keeler, 1989). It is a clonally propagated, late maturing, male sterile variety, grown as an annual with tubers from the previous year's crop serving as propagules. In some agricultural settings 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 and/or Canada (Holm et al., 1991; Muenscher, 1980; 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 become more "weedy" than nontransformed Russet Burbank potato or other potatoes. Resistance to CPB does not appear to be a critical factor determining weediness in Solanaceous species (see Determination for a full discussion). 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; p 27). 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). The susceptibility of Russet Burbank potatoes to many potato diseases (Thompson, 1987) will also limit their competitiveness or persistence as a weed.

More importantly, Monsanto presented field data indicating that CPB-resistant potatoes are no more likely than nontransgenic control Russet Burbank 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, covering 34 field locations at which the CPB-resistant potatoes were evaluated, indicated no obvious differences in the number of volunteers, emergence from seed potatoes, and disease and insect susceptibility (other than to CPB) (Petition, p. 39 and Appendices 5 & 6). Data from field experiments conducted in three geographically diverse potato production areas indicated that CPB-resistant potatoes do not have an increased ability to become weeds by overwintering in cultivated potato-producing areas (Petition, p 349). Percent stand (emergence) and yield of potatoes may be indicators of the fitness and number of potential propagules available to volunteer. No significant differences were observed in these parameters between CPB-resistant potatoes and nontransgenic Russet Burbank in field trials in Idaho and Washington (Petition, pp 178-179).

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 Russet Burbank 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 (see Determination for more specific details). Barriers to gene introgression into cultivated potatoes include the following: (1) male sterility of Russet Burbank (McLean and Stevensen, 1952) and CPB-resistant potatoes would prevent outcrossing; (2) early abscission of flowers, also characteristic of Russet Burbank (McLean and Stevensen, 1952) would limit the success of CPB-resistant potatoes as a female parent; (3) the low acreage planted in male-fertile cultivars reduces the availability of pollen; (4) pollinators (primarily bumblebees) are not attracted to most cultivated potato varieties due to male-sterile flowers and lack of nectar (Petition, pp 324-326; Helgeson and Davies, 1991; and Plaisted, 1980); and (5) cross-pollination with male fertile varieties 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 unable to outcross to male-fertile potato cultivars, and the chances for successful cross-pollination of CPB-resistant potatoes by male-fertile potato cultivars and subsequent seed production will be minuscule. Unless in the later case the progeny are male fertile, there will be no further introgression. Introgression into a male-fertile cultivar would be unlikely to impact genetic diversity of cultivated potatoes in the U.S., since these are vegetatively propagated mostly from certified seed potatoes that are grown under conditions to insure genetic purity (personal survey of seed certification officers). Any transgenic seedlings would be unlikely to persist in the environment because of cultivation and/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 in Section V.A. 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. Two articles submitted in the Petition sufficiently address these issues: Chapter II (Petition, pp. 15-20) by Dr. S. Love, Associate Professor, University of Idaho; and Appendix 12 (also published by Love, 1994). Both of these articles reach the same conclusion discussed below.

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 (see Determination, Section IV.C.2. for species). 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 (see Determination for a more thorough discussion). No natural hybrids have been observed between these species and cultivated potatoes in the U.S.

These barriers, together with the barriers described for Russet Burbank (Section V.B.1. above) 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 appear 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 (see Determination, Section IV.C.3., for a full discussion). 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 only to be cultivated where CPB is a serious pest and in environments suitable to Russet Burbank. 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. His analysis is discussed more fully in the Determination. 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 described for Russet Burbank in Section V.B.1. above. 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 (see Determination for a more thorough discussion). Therefore, APHIS concludes that the possibility for introgression of Monsanto's 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, because these issues have been, or are currently being, addressed by the EPA and FDA as discussed in Section II.C. of this EA. 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 Russet Burbank potatoes.

Russet Burbank potatoes are used for baking, for the manufacture of potato granules and flakes, and for French fries and potato chips (Thompson, N.R., 1987). APHIS considered data supplied by Monsanto on the nutritional constituents, proximate composition (protein, fat, ash, total dietary fiber, carbohydrate, and calories), internal quality characteristics (hollow heart and brown center, internal brown spots, vascular discoloration, and blackspot

bruise), and French fry quality characteristics of CPB-resistant potato tubers compared with those of Russet Burbank tubers. Values for the individual CPB-resistant potato lines (though they varied somewhat) were well within the normal range observed for Russet Burbank tubers (Petition, Tables V.8-V.11, pp. 46-49). Minor differences in some internal quality characteristics can be influenced dramatically by climatic and other growing conditions which favor rapid initial tuber development (Harris, 1992; Burton, 1989). Any lines showing a consistently higher level of internal quality defects would most likely not be further selected for commercialization. The CPB-resistant potatoes were also sufficiently disease and insect free to pass seed certification programs in Idaho, Maine and North Dakota (Petition, Appendix 7, pp. 286-299).

Based on these analyses, APHIS concludes that CPB-resistant potatoes are unlikely 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's 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 Russet Burbank (Petition, Appendix 1, p. 98). 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 Russet Burbank 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 Russet Burbank 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 (Petition, Appendix 10, p. 316). The results showed that Collembola populations were higher in CPB-resistant potato plots and plots of Russet Burbank 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 Monsanto's 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 (Petition, pp.60-62 and 93-94). 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) (Petition, Table VI.1, p. 93). 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 Monsanto's study.

2) Potential impact on threatened and endangered arthropods

The host ranges and habitats of the nine coleopteran insect species currently listed or proposed as threatened and endangered in the U.S. were examined to

USDA/APHIS Petition 94-257-01 for Determination of Nonregulated Status for Colorado Potato Beetle-Resistant Potato Lines BT6, BT10, BT12, BT16, BT17, BT18, AND BT23

Environmental Assessment and
Finding of No Significant Impact

March 2, 1995

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 Russet Burbank potato lines designated BT6, BT10, BT12, BT16, BT17, BT18, and BT23. APHIS received a petition from the Monsanto Company regarding the status of these lines as regulated articles under APHIS regulations at 7 CFR Part 340. APHIS has conducted an extensive review of the petition and supporting documentation, as well as other relevant scientific information. Based upon the analysis documented in this environmental assessment, APHIS has reached a finding of no significant impact on the environment from its determination that Colorado potato beetle-resistant Russet Burbank potato lines BT6, BT10, BT12, BT16, BT17, BT18, and BT23 shall no longer be regulated articles.

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U.S. Department of Agriculture
Date: March 2, 1995

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 (BBEP-EAD National Endangered Species Database, 1994).

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 (Petition, p. 61). 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 and the Development of Insect Resistance to the Btt Insect Control Protein

APHIS considered the potential impacts associated with the cultivation of CPB-resistant potatoes on current agricultural practices used to control CPB, and the potential impacts associated with development of resistance of CPB to the *Btt* insect control protein expressed in these plants and contained in foliar microbial *Btt* insecticides currently registered for use on CPB. Two articles included in the Petition discuss the impact of CPB-resistant potatoes on potato pest management (Petition, p. 65-73 , and pp. 74-90). Monsanto's strategy for maximizing the utility of these plants and delaying the development of insects resistant to the *Btt* insect control protein (Petition, Appendix 9, pg 306) is outlined in the Determination. Their strategy was also submitted to the EPA in support of the registration of the CryIIIA protein as expressed in CPB-resistant potatoes as a plant-pesticide. APHIS reviewers met with EPA's Pesticide Resistance Management Workgroup to discuss their evaluation of this strategy and offer comments. Since this evaluation has been made available by the EPA for the Scientific Advisory Panel meeting (Matten, EPA, 1994), the details will not be presented by APHIS. The development of effective resistance management strategies is an ongoing process, and APHIS will continue to offer comments and suggestions to the EPA and Monsanto to assist in this process. The EPA has stated that they are committed to working with Monsanto to develop product labels and informational brochures that include instructions on the proper use of the CPB-resistant potatoes consistent with resistance management. For a full discussion of current agricultural practices used to control CPB and the impact of CPB-

resistant potatoes, see both the Response to Comments and Section IV.F. of the Determination.

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 (Bob Torla, EPA, Office of Pesticides Programs, personal communication; 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 (R. Roush, W. Tingey, entomologists, Cornell University, personal communication). 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 (Petition, pp. 66-90). 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 (D. Edwards, EPA, personal communication; Rawlings, 1995).

There are currently no commercially available potato cultivars that are resistant to CPB. If commercialized, Monsanto's 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. By the same token, widespread and inappropriate use of <u>either</u> CPB-resistant potatoes or foliar microbial Btt products <u>can</u> and <u>will</u> most likely accelerate the appearance of CPB populations resistant to the *Btt* insect control protein. The rate with which resistance will develop using either approach is difficult to predict because it depends on many assumptions regarding resistance management strategies and their acceptance and effective implementation by growers, the genetics of CPB resistance to this insecticide, and population and behavioral biology of CPB (Roush, 1994; Tabashnik, 1994a and b; Gould et al., 1994). The lack of field-selected resistant CPB populations precludes the direct testing of the validity of models to predict the rate with which CPB will develop resistance using different management strategies. CPB strains selected under laboratory conditions to be resistant to *Btt* sprays do not reach reproductive maturity when fed CPB-resistant potatoes, therefore mechanisms for resistance to sprays may be different from those that might be

effective for resistance to CPB-resistant potatoes (Roush, 1994).

The implementation of an active resistance management plan that is scientifically sound and acceptable to growers should delay the onset of resistance and provide alternative strategies and methods for managing or containing resistant populations if and when they occur. For example, it may be possible to control resistant CPB populations by the use of alternative cultural control practices and alternate insecticides, particularly those to which CPB have not yet been exposed. If resistant populations persist, insecticides based on the *Btt* insect control protein would no longer be effective for controlling CPB on potatoes or on other crops for which these insecticides are registered, such as eggplant and tomato. APHIS has considered: 1) the geographical location where the CPB-resistant potatoes are likely to be grown, 2) the major production areas for tomatoes and eggplants that are subject to CPB pressure, 3) the usage of these insecticides on these crops, and 4) the availability of alternative CPB control measures. These are discussed in detail in Section IV.F. of the Determination.

Based on this analysis, APHIS concludes that there is unlikely to be any significant adverse impact on agricultural practices associated with the appropriate use of CPB-resistant potatoes. Resistance development in insect pest populations is a risk associated with the deployment of insecticides. But in this respect, cultivation of CPB-resistant potatoes 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 Russet Burbank and other potato cultivars. Monsanto has stated that it is in their best interest to delay resistance. The EPA has stated that they will work with Monsanto to develop product labels and informational brochures that are consistent with resistance management, and this should help define the appropriate use of these potatoes. Should resistant CPB populations evolve, it may be possible to limit the persistence and spread of resistant populations. But as with conventional insecticides, where resistance develops, growers will lose the capability to use Btt insecticides to control CPB on potato, and potentially tomato and eggplant. Since these insecticides are currently used infrequently in the major areas of production for these crops, and other options exist for the control of CPB, the impact should be minimal.

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, quality and French fry characteristics to nontransgenic Russet Burbank 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.

6. Development of resistance to insecticides is a potential risk associated with their use; but in this respect, cultivation of CPB-resistant potatoes 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 concludes that CPB-resistant potatoes will be just as safe to grow as Russet Burbank 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

BBEP-EAD National Endangered Species Database. 1994. Administered by Ron McClendon, USDA, APHIS, BBEP-EAD. June 29, 1994.

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

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LIST OF ABBREVIATIONS

CPB	Colorado potato beetle, *Leptinotarsa decemlineata* (Say)
Btt	*B. thuringiensis* subsp. *tenebrionis*
NPTII	neomycin phosphotransferase type II
ELISA	Enzyme-linked immunosorbent assay