RESPONSE TO MONSANTO COMPANY PETITION FOR DETERMINATION OF NONREGULATED STATUS FOR COLORADO POTATO BEETLE RESISTANT POTATO LINES BT6, BT10, BT12, BT16, BT17, BT18, AND BT23.

Prepared by
United States Department of Agriculture
Animal and Plant Health Inspection Service
Biotechnology, Biologics, and Environmental Protection

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

TABLE OF CONTENTS

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 (*Leptinotarsa decemlineata*)-resistant Russet Burbank potato lines BT6, BT10, BT12, BT16, BT17, BT18, and BT23 (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 determination by APHIS has been made in response to a petition received from Monsanto Company (Monsanto) dated September 14, 1994. 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 December 2, 1994, APHIS announced receipt of the Monsanto petition in the Federal Register (59 FR 61866-61867) and stated that the petition was available for public view. APHIS invited written comments on this proposed action, to be submitted on or before January 31, 1995.

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 Russet Burbank variety was genetically engineered by introducing a modified gene encoding a crystalline delta-endotoxin CryIIIA protein, conferring CPB resistance, originally isolated from the soil bacterium *Bacillus thuringiensis* subsp. *tenebrionis* (*Btt*), and a selectable marker gene encoding neomycin phosphotransferase, originally isolated from E. 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 a nonpathogenic organism, *Pisum sativum* (pea).

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. 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 1991 to 1994. 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 has been 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 Russet Burbank 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; 44 FR 50381-50384; and 44 FR 51272-51274). 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 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. The FPPA gives USDA the authority to regulate plant pests and other articles to prevent direct or indirect injury, disease, or damage to plants and plant products. In addition, the PQA provides an additional level of protection by enabling USDA to regulate the importation and movement of nursery stock and other plants that may harbor injurious pests or diseases.

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" for field testing 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*. APHIS believes it prudent to provide assurance before commercialization that organisms such as CPB-resistant potatoes, that are derived at least in part from plant pests, do not pose any potential plant pest risk. Such assurance may aid the entry of new plant varieties into commerce or into breeding and development programs. 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.

The fact that APHIS regulates genetically engineered organisms having plant pest components does not carry with it the presumption that the presence of part of a plant pest makes a whole plant a pest or that the plants or genes are pathogenic (McCammon and Medley, 1990). APHIS' approach to plant pest risk is considerably broader than a narrow definition that encompasses only plant pathogens. Other traits, such as increased weediness, and harmful effects on beneficial organisms, such as earthworms and bees, are clearly subsumed within what is meant by direct or indirect plant pest risk. In APHIS' regulations at 7 CFR Part 340, a "plant pest" is defined as: "Any living stage (including active and dormant forms) of insects, mites, nematodes, slugs, snails, protozoa, or other invertebrate animals, bacteria, fungi, other parasitic plants or reproductive parts thereof; viruses; or any organisms similar to or allied with any of the foregoing; or any infectious agents or substances, which can directly or indirectly injure or cause disease or damage in or to any plants or parts thereof, or any processed, manufactured, or other products of plants."

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. However, because the CPB-resistant potatoes are male sterile, and it is difficult to produce true seed from these plants, APHIS does not anticipate that there will be new potato varieties bred from these CPB-resistant potatoes.

B. **EPA and FDA regulatory authority.**

The CPB-resistant potato lines are currently subject to regulations administered by the EPA and/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 61 comments on the subject Petition from the following categories of respondents: potato farmers (17); universities (10); registered dietitians (9); regional and national potato growers' associations, councils, and boards (5); cooperative extension service (3); State departments of agriculture (3); high school educators (2); individuals (2); potato marketing services (2); potato research company (2); agricultural experiment station (1); department of agriculture of a foreign government (1); food company (1); international technology transfer agency (1); potato processor (1); and a member of the U.S. Congress (1). Fifty-eight of the commenters either expressed support for the petition or provided information in support of nonregulated status for the CPB-resistant potato variety. Among the general points made by the commenters urging approval of the petition were: the need for the product because of the critical problem of CPB to growers; anticipated reduction in pesticide use and resulting public health and environmental benefits; and increased ability to utilize integrated pest management and biological control methods with the new potato variety. These points are discussed in more detail in Section IV. F. of this Determination. The specific points stressed by commenters providing information in support of the petition included an absence of plant pest risk based on the biology of potato and personal observations in field tests with the subject potato lines, the advantages resulting from the specificity of action of the CPB resistance factor, and discussions (by entomologists) of successful resistance management strategies.

Three of the 61 commenters did not directly or indirectly support approval of the petition. One of the three did not address the APHIS approval process; another endorsed the concept of the development of a CPB-resistant potato variety but expressed concern about the development of CPB resistance to Btt.

Only one commenter, an entomologist, urged APHIS to continue to regulate the CPB-resistant potatoes because the commenter felt that they present ".. a risk from plant pests", that risk being "the selection of populations of CPB that are resistant to insect control proteins from *B. thuringiensis* subsp. *tenebrionis*, and thus even more difficult to control...". The commenter suggested that the this risk might be avoided through careful planning of the deployment of CPB-resistant potatoes, and that the planning should include growers who currently use foliar sprays of *Btt* products for control of CPB, because their pest control strategies would be severely affected by the development of resistance. Microbial Btt products were cited as being a critical element of current integrated pest management programs (IPM) in the Northeast and as being increasingly more important in the Midwest and Southeast. The commenter argued that the constitutive high dose expression of the *Btt* insect control protein in the potato foliage throughout the growing season will provide a higher selection pressure for resistance than the use of microbial *Btt* products, which in IPM programs are applied only during the maximum period of effectiveness, i.e., when the majority of first generation larvae are hatching. The high dose strategy is based on the notion that the dose of the *Btt* insect control protein is high enough to control CPB heterozygous for resistance alleles, assuming that resistance is the result of a single major gene that is inherited as a recessive or co-dominant trait. The commenter cites genetic modelling predictions by Gould et al.(1994) that suggest that the number of generations it will take for resistance to develop using this strategy is highly sensitive to small errors in estimates of gene frequency and mode of inheritance. The commenter notes that "Gould also points out that if a CPB population is exposed to selection from low or moderate levels of the insect control protein for any reason (e.g. if environmental factors affect the level of expression, or if farmers use foliar *Btt* sprays on normal potatoes, or if other companies produce engineered lines with lower or more variable levels of expression), this strategy will not be effective".

APHIS fully acknowledges the potential for the selection of CPB resistant to the *Btt* insect control protein expressed in CPB-resistant potatoes. In Section IV. F. of this Determination, APHIS specifically addresses the potential impacts on current agricultural practices used to control CPB in potatoes, as well as in other crops. APHIS also believes that the risks posed by resistance development can be avoided or minimized through careful planning of the deployment of CPB-resistant potatoes. We feel that this condition will be met, and therefore do not feel that continued regulation by APHIS is warranted. APHIS reviewers have met on numerous occasions with members of the EPA to discuss the deployment of the subject potato lines. We have carefully evaluated the resistance management plan (discussed further in section IV.F.) that Monsanto (and their wholly-owned subsidiary, NatureMark) have developed and submitted both to APHIS and the EPA. APHIS and the EPA acknowledge the sensitivity of the high dose strategy to slight differences in the frequency of resistance alleles and their mode of inheritance. However, it is important to note that the high dose strategy should be more effective when used in conjunction with refugia (Roush, 1994, Tabashnik, 1994). Refugia is the use of untreated refuge plants to maintain insects that are susceptible to the insecticide, and which will interbreed with potentially resistant insects. This serves to dilute the frequency of resistance alleles in the population, thereby delaying resistance. Monsanto's resistance management plan includes among other things, the use of refugia and IPM. APHIS also wishes to clarify that their determination that CPB-resistant potatoes should no longer be subject to regulation under 7 CFR Part 340, in no way releases them from regulations administered by the EPA under FIFRA and FFDCA as described in the Background (Section II above).

APHIS also received comments from five other entomologists who acknowledged the potential risk of resistance development, but who still support a determination of nonregulated status for the CPB-resistant potatoes. They also noted that Monsanto has demonstrated commendable concern for this issue by supporting and coordinating research by several entomologists to help devise the best strategy for avoiding resistance. One commenter noted that "An extensive research program was initiated in 1990 involving scientists in WI, OH, NY, MD, MI, NC, Ontario, New Brunswick and other locations and a comprehensive database on CPB resistance management and transgenic *Btt* has already been developed. The database includes the relevant molecular, biological, behavioral, ecological and agronomic information required to develop specific and practical deployment strategies for transgenic potatoes to prevent resistance development." Another commenter noted that variations in baseline susceptibilities of CPB to the *Btt* insect control already measured in 60 CPB populations in potato growing areas have not been correlated to *Btt* usage, and that NatureMark is committed to continued resistance monitoring after commercialization in order to detect and mitigate early stages of resistance development in individual populations. Another commenter noted that the use of transgenic potato plants with the resistance management strategies being developed would not result in greater risk of resistance than regular use of foliar *Btt* sprays, and that the best way to avoid resistance is to have a large arsenal of different control measures (including CPB-resistant potatoes) that are used strategically in combination. Their comments also cited many of the benefits offered by the use of CPB-resistant potatoes (also described in Section IV. F.), including a reduction in the use of broad spectrum insecticides and the delay in resistance to new insecticides with a different mode of action.

APHIS appreciates these comments, and concurs with the conclusion that the CPB-resistant potatoes should no longer be subject to regulation under 7 CFR Part 340.

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). *Solanum tuberosum* cv. Russet Burbank is the most widely planted cultivar in the United States (National Potato Council, 1994).

Potato belongs to the family Solanaceae which has about 90 genera and 2,800 species and which also includes 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 a herbaceous, clonally propagated crop grown as an annual. The flowers are perfect (containing both pistils and stamens), but are typically outcrossed, and require insects for pollination, in particular bumblebees. The fruits are berries, but are absent in many cultivars such as Russet Burbank (Burbank, 1921) due to many factors which can block successful fertilization and/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 Russet Burbank cultivar 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* (*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. *Btt* is not a regulated article. 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. The gene has no involvement in plant disease or damage. This gene was introduced as a 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 a nonpathogenic organism, *Pisum sativum* (pea), and two organisms which are plant pathogens: the bacterium *A. tumefaciens* and CaMV. Specifically, the DNA regulatory sequences associated with the *cryIIIA* gene comprise the promoter derived from the 35S gene of CaMV with a duplicated enhancer region (Kay et al., 1987; Odell et al., 1985) 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. The genes and their regulatory sequences were subcloned between the left and right T-DNA borders of an *A. tumefaciens* binary-plasmid transformation vector designated PV-STBT02.

CPB-resistant potato plants were derived by transforming Russet Burbank stem sections with PV-STBT02 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 (see reviews by 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. However, as noted above, both transformed and nontransformed Russet Burbank potato plants are male-sterile.

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

Determination

accumulation of the proteins encoded by the introduced genes for each of the transformed lines grown at seven potato production sites in the U.S. was determined by enzyme-linked immunosorbent assays (ELISA) (Petition, pp. 36-45). *Btt* insect control protein in early-season leaf tissue (nine weeks post-planting) ranged from 0.20 to 30.80 μg/g fresh weight with a mean of 19.1 across all seven lines and all sites. Means were only slightly lower at twelve and fifteen weeks post planting. Mean values in whole plant tissue across all lines and sites taken early in the season and at the onset of senescence were 5.41 and 6.64 μg/g fresh weight, respectively. The mean accumulation in tubers at harvest time across all lines and sites was much lower (1.01 μg/g fresh weight) and ranged from 0.28 to 2.00 μg/g fresh weight. Compared to *Btt* insect control protein, the mean accumulation of NPTII protein across all lines and sites was lower.

During extensive field testing, the CPB-resistant potatoes exhibited the typical agronomic characteristics of the recipient Russet Burbank variety, 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 Russet Burbank 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, 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 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 by some ecologists as nonpredictive, 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, particularly the Russet Burbank variety, lacks most of Baker's "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 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 and/or Canada in most weed compendiums (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 Russet Burbank or other potato cultivars. None of the characteristics of weeds described by Baker involved resistance or susceptibility to insects. Resistance to CPB does not appear 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 data base 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 Russet Burbank 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.

More importantly, in addition to the analysis above, APHIS evaluated field data submitted by Monsanto which specifically demonstrates that CPB-resistant potatoes are no more weedy than the non-modified recipient. Control and CPB-resistant potato plants were routinely monitored during field trials for differences in physical characteristics (plant vigor, plant height, chlorotic color, leaflet shape, and flowering), disease susceptibility (early blight, late blight, leaf spot, rusts, *Verticillium*, mildew, potato leaf roll virus, and potato virus Y), and insect susceptibility (aphids, CPB, cutworms, leafhoppers, and spider mites). The field data reports, covering 34 field locations at which the CPB-resistant potatoes were evaluated in 1991-1993, indicated no obvious differences compared to nontransgenic control Russet Burbank 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).

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 Russet Burbank potatoes (Petition, p. 349). 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 (Petition, pp 178-179) 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 Russet Burbank variety.

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 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 variety Russet Burbank is male sterile (it produces no pollen) (McLean and Stevensen, 1952, Robert Hanneman, Research Geneticist, USDA, ARS, Department of Horticulture, University of Wisconsin, personal communication). As stated in Section IV., Monsanto reported no obvious differences in the flowering of CPB-resistant potatoes compared to the nontransgenic Russet Burbank. There is no reason to believe that the genetic construct introduced during the transformation event would have any effect on the reproductive biology of the CPB-resistant potatoes, unless the insertion event interrupted a genetic locus critical for the determination of normal reproductive function.

Therefore, the only pathway for sexual gene flow would be for the CPB-resistant potatoes to be used as a female parent in a cross with a male-fertile sexually-compatible species. The success of such a cross would be limited by early abscission of flowers, also characteristic of Russet Burbank (McLean and Stevensen, 1952). If progeny were produced from such a cross, and the progeny were male-sterile, then transfer of genes would be impossible. If the progeny were male fertile, then this could lead to outcrossing of the trait to other sexually-compatible species. McLean and Stevensen (1952) describe some artificial methods and natural conditions (i.e., stem girdling resulting from *Rhizoctonia solani* infection) whereby seed can be produced on Russet Burbank following pollination by male-fertile potato cultivars. Russet Burbank (as well as other cultivars) are generally not cultivated with different cultivars simultaneously in the same field, but overwintering volunteers of one variety may emerge in a field newly planted in another variety, presenting an opportunity for cross-pollination. However, the probability of this occurrence is low due to the relatively low acreage planted in male-fertile cultivars. Of fall potatoes planted in 1993, Russet Burbank is listed as one of the two top varieties planted in eight of the eleven major states in the U.S.(NPC, 1994). Many of the most popular cultivated potato varieties grown in the U.S. and Canada have little or no pollen (NPC, 1994; Petition, p 324). In some of these varieties, seed production is promoted in part by cool, summer night temperatures. 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 ~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 cultivars under production, low pollen dispersal rates of male-fertile potato varieties, lack of incentive for insect pollination, and high flower abscission rates in CPB-resistant potatoes, the chances for successful cross-pollination of CPB-resistant potatoes by male-fertile potato cultivars will be minuscule. 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 can be planted (personal survey of seed potato certification officers). 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 and/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 (Petition, p. 16). 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 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. Monsanto submitted as part of its petition two articles that sufficiently address these issues: *The Potential for Gene Escape from Cultivated Transgenic Potatoes Within the U.S.* by Dr. Steve Love, Associate Professor, University of Idaho (Petition, pp. 15-20); and Appendix 12- *Ecological Risk of Growing Transgenic Potatoes in the United States and Canada: Potential for Vegetative Escape or Gene Introgression into Indigenous Species* by Drs. S. L. Love and J. J. Pavek, University of Idaho. The latter article has since been published by Love under the same title in the American Potato Journal (Love, 1994). Both of these articles reach the following conclusions.

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*).

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.

The articles conclude that these barriers, along with other barriers to introgression from cultivated potatoes (described for Russet Burbank in Section IV.C.1. above), insure that gene introgressio_wfrom transgenic cultivated potatoes into free-living tuber-bearing *Solanum* species in the U.S is impossible or highly 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 extremely 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, since 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 Russet Burbank 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 and in Central America (including 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 (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 or 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; Edward B. Radcliffe, entomologist, Department of Entomology, University of Minnesota, St. Paul, Minnesota, personal communication). 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)._wBut 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. Russet Burbank is generally not grown in Mexico anyway (Dick Casagrande, entomologist, Rhode Island, personal communication; and Terry Stone, Monsanto, personal communication). Introgression in all of these cases would be further limited by those barriers described in Section IV.C.1. above.

APHIS has concluded 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 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 (Dick Casagrande, entomologist, University of Rhode Island, personal communication; Logan and Lu, 1993). For this reason, growers in this region would have no incentive to grow Monsanto's CPB-resistant potatoes unless CPB populations there change their host preference to cultivated potatoes or the Cry IIIA insect control protein is efficacious in controlling other significant pests of potatoes which occur there.

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.

Determination

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 French fry 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. 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).

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 and other processed potato products (Thompson, 1987). APHIS evaluated data supplied by Monsanto comparing the nutritional constituents and quality attributes of CPB-resistant potato tubers with those of nonmodified Russet Burbank tubers. Data on the most important potato constituents collected from field trials conducted in a total of six potato production locations indicated significant differences in percent dextrose and glycoalkaloid content for some of the CPB-resistant potato lines compared to Russet Burbank controls; however, the values for these traits, as well as those for total solids, sucrose, vitamin C, and protein, were well within the normal range observed for Russet Burbank tubers (Petition, Table V.8, p. 46). Glycoalkaloids at excessive levels are associated with undesirable flavor and mammalian toxicity and teratogenicity (Gregory et al., 1981). Data on quality characteristics (Petition, Table V.9-V.10, pp. 47-48) and proximate composition (Petition, Table V.11, p. 49) were collected from field trials conducted in two locations. Results indicated significant differences in two associated internal physiological defects, hollow heart and brown center, for only two of the CPB-resistant potato lines compared to Russet Burbank controls. But such minor differences in these defects are considered common among potato varieties and are influenced dramatically by climatic and other growing conditions which favor rapid initial tuber development (Harris, 1992; Burton, 1989). If these lines continued to exhibit this defect, they would most likely not be marketed. CPB-resistant potato tubers were not significantly different from the controls for the other internal quality characteristics (internal brown spots, vascular discoloration, and blackspot bruise), French fry quality characteristics, or in proximate composition (protein, fat, ash, total dietary fiber, carbohydrate, and calories).

The CPB-resistant potatoes were also entered into seed certification programs in Idaho, Maine and North Dakota, and were screened for the absence of many common diseases and insects. They were granted certification for current season and post harvest evaluation (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.

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, since 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 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.

In Oregon, CPB-resistant potatoes with no treatment or with systemic insecticides for aphid control were compared to Russet Burbank 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 Russet Burbank 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 and Russet Burbank both untreated or treated with systemic insecticide for control of CPB, potato flea beetle and aphids. Table 1 describes these treatments in more detail. 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 Russet Burbank 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 and Russet Burbank 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 Russet Burbank 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 Russet Burbank, 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 Russet Burbank plots. Even though adult potato flea beetle populations were not reduced, significantly reduced feeding damage compared to untreated Russet Burbank 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 (Petition, Appendix 10, p. 316). The results showed that Collembola populations were higher in plots containing untreated CPB-resistant potatoes or Russet Burbank treated with microbial *Btt* pesticide than in plots untreated or treated with conventional systemic insecticide.

Determination

Table 1. The insecticide treatments and their respective target pests at each location for each cultivar, ie., nonmodified Russet Burbank (NRB) or CPB-resistant potatoes (CPB-RB), are shown.

Location	Cult.	Insecticide Treatment	Target Pests
OR	NRB	•Broad spectrum, permethrin foliar spray	•CPB
	NRB	Systemic, phorate & disulfoton	CPB + Aphids
	CPB-RB	•Systemic, phorate & disulfoton •Endogenous *Btt* protein	•CPB + Aphids CPB
	CPB-RB	Endogenous *Btt* protein only	CPB
	NRB	Microbial *Btt*[1]	CPB
	NRB	None	
WI	NRB	Conventional foliar-applied: •Esfenvalerate •Endosolfan •Methamidophos	 •CPB larvae •CPB summer adults •PLH[2] & Aphids
	CPB-RB	•Endogenous *Btt* protein •Foliar-applied malathion	•CPB larvae & adults •Selectively applied for PLH
	NRB	•Microbial *Btt*+ Esfenvalerate •Foliar-applied malathion	•CPB larvae & adults •Selectively applied for PLH
	NRB	Foliar-applied malathion	Selectively applied for PLH
PEI	NRB	Systemic phorate	CPB, PFB[3], Aphids
	CPB-RB	•Endogenous *Btt* protein •Systemic phorate	•CPB larvae & adults •PFB, Aphids
	CPB-RB	Endogenous *Btt* protein only	CPB larvae & adults
	NRB	None	

[1]The microbial *Btt* product used was M-trak● [EPA registration no. 53219-2], which is a soluble concentration of the *B.t. san diego* delta endotoxin encapsulated in killed *Pseudomonas fluorescens* bacteria. The *B.t. san diego* and *Btt* strains and their delta-endotoxin *cryIIIA* genes are indistinguishable (MacIntosh et al., 1990).

[2]PLH=Potato leafhopper

[3]PFL=Potato flea beetle

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'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 evaluated the results of those 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 (*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 (Petition, Table VI.2, p. 94). 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), (Petition, Table VI.1, p. 93). 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's 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

Determination

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 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.

F. 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 considered potential impacts associated with the cultivation of CPB-resistant potatoes on the current agricultural practices used to control CPB. APHIS also considered potential impacts associated with the appearance of CPB resistant to the *Btt* insect control protein expressed in these plants and contained in foliar microbial *Btt* insecticides currently registered for CPB control on potato. Two articles included in the Petition discuss the impact of CPB-resistant potatoes on potato pest management: 1) *Transgenic Host Plant Resistance and Insect Management in Potatoes*, by R. Roush, Cornell University (Petition, p. 65); and 2) *Impacts of Transgenic Host Plant Resistance to Colorado Potato Beetle on Potato Culture in the United States*, by J. Wyman, University of Wisconsin (Petition, p. 74). Monsanto also submitted their 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). Their strategy was also submitted to the EPA in support of the registration of the these 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 of that evaluation 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 recommendations for technical improvements 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 that are consistent with resistance management.

1) Current agricultural practices used to control CPB on potatoes.

CPB is the predominant defoliating pest causing economic damage to potato crops in the U.S., particularly in the eastern and north central production areas where control costs frequently exceed $200.00/A. The potato leafhopper, potato flea beetle, green peach aphid and potato aphid are also serious pests in U.S. potato production. The Russet Burbank potato variety is predominantly grown in the north central and north western production regions, and is the second major variety of fall potatoes planted in Maine (National Potato Council, 1994). To what extent the CPB-resistant Russet Burbank potato lines will be cultivated in the eastern region is not known. In New York, this variety does not perform well; and Atlantic, Superior, and Monona are the most popular varieties (Bob Plaisted, plant breeder, Cornell University, personal communication). Most CPB control methods are primarily directed at controlling early season adults and the first generation larvae (Petition, pp. 66-90). Both larvae and adult CPB can cause severe damage to potato crops. There are usually 2-3 generations of CPB per year, and the overwintering adult survival rate is high (60%). Newly emerged potato plants can suffer severe damage from adults that emerge from the soil in nearby fields and crawl (or fly) into newly planted potato fields in the early spring.

Cultural control practices include: 1) luring adult beetles onto trap crops planted at the edge of overwintering habitat and then destroying the insects by flame, pesticide, or other means; 2) the placement of plastic-lined trench traps between the overwintering habitat and new crop to catch adults; 3) flaming young plants with a propane torch on a 3-4 day schedule; 4) crop vacuums; 5) destruction of overwintering habitat; and 6) crop rotation (Petition, pp. 66-90). Many of these methods are limited by the necessity for proper climatic conditions. Some methods, such as crop vacuums and propane flamers, can kill beneficial insects (such as the lady beetle, *Coleomegilla maculata*, which feeds on CPB egg masses). In a 1992 New York potato grower survey, propane flaming, trap crops, and trench traps were used for CPB control on approximately 12%, 1.6%, and 0.75%, respectively, of the total potato acreage planted. Crop rotation is generally only practiced on large farms and is effective when the nearest potato field planted the previous year was at least 1 mile away.

Biological insecticides such as rotenone and foliar microbial *Btt* products are primarily effective at controlling young larvae. The *Btt* products have been available since the late 1980's, and no field resistance has been reported. These biological insecticides are also limited by poor performance under cool temperatures, short field persistence, and the necessity for proper weather and soil conditions for application. Newer *Btt* formulations have improved persistence and efficacy. In New York approximately 27-58% of the acres planted in potatoes in 1993-1994 were treated with *Btt* insecticide formulations (R. Roush, W. Tingey, entomologists, Department of Entomology, Cornell University, personal communication; New York Agricultural Statistics Service, 1994). 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). Natural predators are generally not effective at maintaining CPB populations below damaging levels in commercial fields. Natural enemies and predators being explored as biological control agents of CPB include two predaceous stink bugs (*Perillus bioculatus* and *Podisus maculiventris*) and a hymenopteran parasitoid from Columbia (*Edovum puttleri*) (Radcliffe et al., 1991; Bill Cantelo, entomologist, USDA, ARS, Beltsville, Maryland, personal communication). However, the parasitoid does not survive the winter in temperate climates (Raman and Radcliffe, 1992). CPB have been controlled experimentally with other biological control agents, including some entomopathic fungi and nematodes; but none of these has been developed for commercial use (Raman and Radcliffe, 1992).

Conventional chemical insecticides are the primary means of controlling economically damaging densities of CPB populations, and they are the only currently available effective means of controlling 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. In the northeastern region, two kinds of insecticide mixtures, a pyrethroid/piperonyl butoxide combination and an oxamyl/endosulfan mixture, are still effective where resistance has not yet developed; however, groundwater contamination by oxamyl has become a problem. Systemic carbamate insecticides previously used to control CPB have been withdrawn from use for the same reason and because of resistance. The pyrethroids (such as permethrin) are also toxic to many beneficial insect predators. Two new chemical insecticides,i.e. systemic soil and foliar formulations of imidacloprid, have been registered by the EPA for control of CPB and other pests on potatoes (Dennis Edwards, EPA, personal communication; Rawlings, 1995).

2) Potential impact of CPB-resistant potatoes on current agricultural practices.

There are currently no commercial CPB-resistant potato cultivars available. Monsanto's transgenic CPB-resistant potatoes offer an alternative control method. Monsanto's strategies for maximizing the utility of these plants while delaying the development of CPB resistance to these plants include the following:

1) Promoting the incorporation of CPB-resistant potatoes into integrated pest management programs (IPM) that emphasize the use of cultural control practices, such as those described above, and judicious and selective use of additional insecticides only when pest populations reach the threshold for economic damage. They do not encourage the use of CPB-resistant potatoes as a stand-alone control measure.
2) Monitoring CPB populations for *Btt* protein susceptibility so that development of resistance can be detected and management strategies altered accordingly.
3) High dose expression of *Btt* protein to control CPB that are heterozygous for resistance alleles.
4) Deployment of non-CPB-resistant potatoes or other hosts as refugia for CPB that are sensitive to the *Btt* insect control protein, in order to maintain susceptible alleles in the population.
5) Development of new CPB control proteins with a distinct mode of action to be employed with the *Btt* protein.
6) Implementation of a grower education program to achieve items 1, 2, and 4 above.

Determination

APHIS evaluated the potential impact to agricultural practices associated with the use of CPB-resistant potatoes according to Monsanto's strategy. As a result of Monsanto's program to instruct growers on the use of cultural control practices and IPM, growers may be more likely to adopt these methods. However, growers will also need to be informed about the implementation of preferred refugia strategies and how these can be integrated with other cultural practices. Growers will be less likely to use chemical insecticides targeted at CPB control, and this should reduce the risks associated with some of these insecticides. The use of CPB-resistant potatoes should increase safety to field workers and consumers, reduce toxicity to nontarget species, and lower rates of ground water contamination by insecticides such as oxamyl. As described in Section IV.E. above, Monsanto has demonstrated that generalist predators were significantly enhanced in CPB-resistant potatoes not treated with broad-spectrum insecticides. This may help control other potato pests, particularly the green peach aphid, which is a vector for the potato leaf roll virus, a serious virus of potatoes. This could result in a reduction in use of chemical insecticides against aphids and therefore, a delay in the development of resistance to these insecticides. The CryIIIA protein, as expressed in CPB-resistant potatoes, appears not to be toxic to Hymenoptera and other nontarget arthropods. Therefore, it should be more compatible than broad-spectrum insecticides with the use of natural enemies and predators being developed for biological control of CPB and for biological control of other potato pests, particularly aphids. Biological control of aphids has been successful in other crops. CPB-resistant potatoes are not likely to eliminate completely the use of chemical insecticides, particularly when they may be needed to control other serious pests such as potato leafhoppers and potato flea beetles. But perhaps they may encourage more selective use of insecticides against these pests.

CPB-resistant potatoes could provide a more flexible and effective alternative for season-long control of CPB compared to the use of some foliar microbial *Btt* products that nationwide currently only receive limited use. Monsanto's field data (see Section IV.E. above) indicate that CPB-resistant potatoes are more effective than some *Btt* formulations at reducing CPB survival and egg-laying. The CPB-resistant potato alternative is particularly important where chemical insecticides are no longer effective due to insect resistance.

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 evolution of resistance to the *Btt* insect control protein in CPB populations. The rate with which resistance will develop using either approach is difficult to predict because it depends on many assumptions and factors relating to the effectiveness of resistance management strategies and their acceptance and effective implementation by growers, the genetics of CPB resistance to these insecticides, and population and behavioral biology of CPB (Gould et al., 1994; Roush, 1994; Tabashnik, 1994a and b). Some have argued that resistance in some cases may develop more quickly with sprays than with transgenic plants (Roush, 1994). Monsanto's support of research in these areas and their grower education plan, coupled with CPB population monitoring programs, could enable them to implement strategies to delay resistance and detect and possibly contain it when it occurs. A five fold range in baseline susceptibility to the *Btt* insect control protein exhibited in the 60 CPB populations assayed to date, is typical of the range in susceptibility of other insects to delta-endotoxin proteins; and there is no evidence indicating that this variation is correlated with the previous use of foliar *Btt* formulations (G. Dively, entomologist, University of Maryland, personal communication). The lack of availability 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 or lay eggs, when larvae or adults, respectively, are fed on potatoes genetically engineered to express the CryIIIA protein (Roush, 1994). Therefore, mechanisms for resistance to sprays may be different than those which will be effective for resistance to CPB-resistant potatoes. Resistance to delta-endotoxin insecticides from other *B. thuringiensis* subspecies has occurred in lepidopteran insect pests of treated stored grain (McGaughey and Beeman, 1988) and in diamondback moths treated in extensive spray programs (Tabashnik et al., 1990; Ferré et al., 1991).

If and when resistance occurs, it may be possible to control resistant CPB populations by the use of cultural control practices and alternate insecticides, particularly those to which CPB have not yet been exposed such as imidacloprid. If resistant populations persist, insecticides based on the *Btt* insect control protein may no longer be effective at controlling CPB on potatoes and other crops for which these insecticides are registered. Such insecticide formulations are currently registered for the control of CPB larvae on eggplant and tomato as well as potato (EPA, 1991 and 1988). CPB prefers potatoes, but in the northeast where CPB populations are higher, eggplants and fresh market tomatoes (particularly in New York and New Jersey) can be subjected to CPB damage (Bill Cantelo, entomologist, USDA, ARS, Beltsville, MD., personal communication; Berlinger, 1986). Russet Burbank is not a major variety planted near those areas (National Potato Council, 1994). In the two major eggplant producing states, New Jersey and Florida, *Bt*-based insecticide usage on eggplant acreage in 1992 was estimated at 13 and 14%, respectively (USDA, National Agricultural Statistics Service, 1993). Most commercial tomato production is not centered around potato production areas that have serious CPB problems (USDA, 1993), and therefore it is likely to be more isolated from resistant CPB populations potentially resulting from the long-term cultivation of CPB-resistant potatoes. Michigan, New York, and New Jersey are the only states that are both listed as major states for either fresh market or processing tomato production and where CPB is considered a serious pest on potatoes. In these states, *Bt*-based insecticide usage on total tomato acreage in 1992 was estimated at 10% in New Jersey and less than 1% for both Michigan and New York (USDA, National Agricultural Statistics Service, 1993); however, these insecticides could include some *B.t.* subspecies *kurstaki* based insecticides, which are used for treating certain lepidopteran pests. Nationwide less than 1% of commercial tomato acreage was treated with *Btt*-based products for CPB control in 1992 (Bob Torla, EPA, Office of Pesticides Programs, personal communication). Other options are currently available for the control of CPB on these crops. Most of the chemical insecticides (i.e., cryolite, rotenone, oxamyl, endosulfan, pyrethroids including permethrin, and some organophosphates) used for control of CPB on eggplant and tomato are the same or similar to those registered for use on potato (University of Maryland, 1994; USDA, National Agricultural Statistics Service, 1993). The hymenopteran parasitoid, *Edovum puttleri*, is also being used to control CPB in eggplant (R. Balaam, New Jersey Department of Agriculture, personal communication).

APHIS concludes that 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. The implementation of an active resistance management plan that is scientifically sound and acceptable to growers should delay the onset of resistance to CPB-resistant potatoes and provide alternative strategies and methods for managing or containing resistant CPB populations if and when they occur. Product labels and informational brochures should help define the appropriate use of these potatoes and reduce any potential risks associated with their use. In those areas where resistant CPB populations evolve and persist, growers could lose the capability to use *Btt*-based insecticides to control CPB as a pest on potato, and potentially on tomato and eggplant. Since these insecticides are currently used infrequently to control CPB in the major areas of production for Russet Burbank and these other crops, and other options exist for the control of CPB, the impact should be minimal.

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 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 control of 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 unlikely to be new varieties bred from CPB-resistant potatoes; however, if such varieties were developed 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 from Russet Burbank in traditional breeding programs.

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