DEPARTMENT OF AGRICULTURE

Animal and Plant Health Inspection Service

[Docket No. 99–036–2]

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

AGENCY: Animal and Plant Health Inspection Service, USDA.

ACTION: Notice.

SUMMARY: We are advising the public of our decision to extend to one additional potato line our determination that certain potato lines developed by Monsanto Company, which have been genetically engineered for insect and virus resistance, are no longer considered regulated articles under our regulations governing the introduction of certain genetically engineered organisms. Our decision is based on our evaluation of data submitted by Monsanto Company in its request for an extension of a determination of nonregulated status, an analysis of other scientific data, and comments received from the public in response to a previous notice. This notice also announces the availability of our finding of no significant impact.

EFFECTIVE DATE: July 17, 2000.

ADDRESSES: The extension request, an environmental assessment and finding of no significant impact, and all comments received may be read at USDA, room 1141, South Building, 14th Street and Independence Avenue SW., Washington, DC, between 8 a.m. and 4:30 p.m., Monday through Friday, except holidays. To be sure someone is there to help you, please call (202) 690–2817 before coming.

FOR FURTHER INFORMATION CONTACT: Dr. James White, Biotechnology Assessments Section, Permits and Risk Assessments, PPQ, APHIS, Suite 5B05, 4700 River Road Unit 147, Riverdale, MD 20737–1236; (301) 734–5940. To obtain a copy of the extension request or the environmental assessment and finding of no significant impact, contact Ms. Kay Peterson at (301) 734–4885; e-mail: kay.peterson@usda.gov.

SUPPLEMENTARY INFORMATION: The regulations in 7 CFR part 340, "Introduction of Organisms and Products Altered or Produced Through Genetic Engineering Which Are Plant Pests or Which There is Reason to Believe Are Plant Pests," regulate, among other things, the introduction (importation, interstate movement, or release into the environment) of organisms and products altered or produced through genetic engineering that are plant pests or that there is reason to believe are plant pests. Such genetically engineered organisms and products are considered "regulated articles."

The regulations in § 340.6(a) provide that any person may submit a petition to the Animal and Plant Health Inspection Service (APHIS) seeking a determination that an article should not be regulated under 7 CFR part 340. Further, the regulations in § 340.6(e)(2) provide that a person may request that APHIS extend a determination of nonregulated status to other organisms. Such a request must include information to establish the similarity of the antecedent organism and the regulated article in question.

Background

On June 22, 1999, APHIS received a request for an extension of a determination of nonregulated status (APHIS No. 99–173–01p) from Monsanto Company (Monsanto) of St. Louis, MO, for a Russet Burbank potato line designated as NewLeaf® Plus line RBMT22–82 (RBMT22–82), which has been genetically engineered for resistance to the Colorado potato beetle (CPB) and potato leaf roll virus (PLRV). Monsanto requested an extension of a determination of nonregulated status issued previously for NewLeaf® Plus Russet Burbank potato lines RBMT21–129 and RBMT21–350, APHIS petition number 97–204–01p (63 FR 69610–69611, December 17, 1998, Docket No. 97–094–2). Based on the similarity of RBMT22–82 to RBMT21–129, the antecedent organism, Monsanto requested a determination that CPB-and PLRV-resistant potato line RBMT22–82 does not present a plant pest risk and, therefore, is not a regulated article under APHIS' regulations in 7 CFR part 340.

On March 6, 2000, APHIS published a notice in the **Federal Register** (65 FR 11758–11759, Docket No. 99–036–1) announcing that an environmental assessment for Monsanto's extension request had been prepared and was available for public comment. During the designated 30-day public comment period, APHIS received 10 comments from the following sources: State potato commissions, a potato growers association, an organic consumers association, the U.S. Department of Agriculture's Agricultural Research Service, a State university, a State university agricultural experiment station, plant virologists, a farmer, and a private individual. Six of the comments were in favor of the extension request, and four were in opposition. A majority of the commenters expressing support for deregulating potato line RBMT22–82 stressed its effectiveness in resisting the damage caused by CPB and PLRV and the associated benefits of reduced pesticide use. Several commenters in opposition to deregulation of the subject potato line expressed concern that insufficient safety testing had been done on such issues as genetic drift, the development of insect resistance, effects on beneficial organisms, and the potential for the development of novel plant viruses through expression of parts of viruses from a transgene. APHIS identified and addressed these issues in the environmental assessment prepared for line RBMT22–82 and in the environmental assessment and determination prepared for the antecedent organism. In consideration of the comments submitted to us, we have included a response to comments as an attachment to our finding of no significant impact (FONSI) for the environmental assessment. The environmental assessment and the FONSI, including the attachment, are available from the person listed under **FOR FURTHER INFORMATION CONTACT.**

Analysis

Like the antecedent organism, potato line RBMT22–82 contains the *cry3A* gene derived from *Bacillus thuringiensis* subsp. *tenebrionis (Btt) and the orf1/orf2* gene derived from PLRV. The *cry3A* gene encodes an insecticidal protein that is effective against CPB and the *orf1/orf2* gene imparts resistance to PLRV. Potato line RBMT22–82 also contains the *CP4 EPSPS* selectable marker gene, while the antecedent organism contained the *nptII* selectable marker gene. The subject potato line and the antecedent organism were developed through use of the *Agrobacterium tumefaciens* transformation system, and expression of the added genes in RBMT22–82 and the antecedent organism is controlled in part by gene sequences derived from the plant pathogens figwort mosaic virus and *A. tumefaciens*.

Potato line RBMT22–82 and the antecedent organism were genetically engineered using the same transformation method and with the same genes that make the plants insect and virus resistant. Accordingly, we have determined that RBMT22–82 is similar to the antecedent organism RBMT21–129 in APHIS petition 97–204–01p and, therefore, should no longer be regulated under the regulations in 7 CFR part 340.

The subject potato line has been considered a regulated article under APHIS' regulations in 7 CFR part 340 because it contains gene sequences derived from plant pathogens. However, evaluation of field data reports from field tests of RBMT22–82, conducted under APHIS permits and notifications since 1994, indicates that there were no deleterious effects on plants, nontarget organisms, or the environment as a result of its environmental release.

Determination

Based on an analysis of the data submitted by Monsanto, a review of other scientific data, and field tests of the subject potato line, APHIS has determined that Russet Burbank potato line RBMT22–82: (1) Exhibits no plant pathogenic properties; (2) is no more likely to become a weed than similar pest-resistant potatoes developed by traditional breeding techniques; (3) is unlikely to increase the weediness potential for any other cultivated or wild species with which it can interbreed; (4) will not cause damage to raw or processed agricultural commodities; and (5) will not harm threatened or endangered species or other organisms, such as bees, that are beneficial to agriculture. Therefore, APHIS has concluded that potato line RBMT22–82 and any progeny derived from crosses with other potato varieties will be as safe to grow as potatoes that are not subject to regulation under 7 CFR part 340.

Because APHIS has determined that potato line RBMT22–82 does not present a plant pest risk based on its similarity to the antecedent organism, Monsanto's potato line RBMT22–82 will no longer be considered a regulated article under APHIS' regulations in 7 CFR part 340. Therefore, the requirements pertaining to regulated articles under those regulations no longer apply to the field testing, importation, or interstate movement of the subject potato line or its progeny. However, importation of potato line RBMT22–82 and seeds capable of propagation are still subject to the restrictions found in APHIS' foreign quarantine notices in 7 CFR part 319.

National Environmental Policy Act

An environmental assessment (EA) was prepared to examine the potential environmental impacts associated with this determination. The EA was prepared in accordance with: (1) The National Environmental Policy Act of 1969 (NEPA), as amended (42 U.S.C. 4321 *et seq.*), (2) regulations of the Council on Environmental Quality for implementing the procedural provisions of NEPA (40 CFR parts 1500–1508), (3) USDA regulations implementing NEPA (7 CFR part 1b), and (4) APHIS' NEPA Implementing Procedures (7 CFR part 372). Based on that EA, APHIS has reached a finding of no significant impact (FONSI) with regard to its determination that Monsanto's potato line RBMT22–82 and lines developed from it are no longer regulated articles under its regulations in 7 CFR part 340. Copies of the EA and the FONSI are available upon request from the individual listed under **FOR FURTHER INFORMATION CONTACT.**

Done in Washington, DC, this 9th day of June 2000.

Bobby R. Acord,

Acting Administrator, Animal and Plant Health Inspection Service.

[FR Doc. 00–15152 Filed 6–14–00; 8:45 am]

BILLING CODE 3410–34–P

Approval of Monsanto Request (99-173-01p) Seeking Extension of Determination of Non-regulated Status For Potato Leaf roll Virus and Colorado Potato Beetle resistant Potato Line RBMT22-82

Finding of No Significant Impact

April 2000

The Animal and Plant Health Inspection Service (APHIS), United States Department of Agriculture (USDA), has prepared an environmental assessment (EA) prior to approving an extension (APHIS Number 99-173-01p) of the determination of nonregulated status granted to Monsanto Company for petition 97-204-01p under APHIS regulations at 7 CFR Part 340. The subject of extension request 99-173-01p is potato line RBMT22-82, which has been genetically engineered with two genes whose expression results in the plant being resistant to potato leaf roll virus and Colorado potato beetle. Based on the analysis carried out in the EA, APHIS has reached a finding of no significant impact (FONSI) to the environment from its determination that potato line RBMT22-82 shall no longer be considered a regulated article. Before reaching this decision, APHIS requested and considered comments on the EA from the public. A response to these comments is included as an attachment to this FONSI statement.

John H. Payne, Ph.D.
Assistant Director
Plant Protection and Quarantine
Animal and Plant Health Inspection Service
U.S. Department of Agriculture
Date: June 6, 2000

Attachment
Finding of No Significant Impact
Response to Comments
APHIS no. 99-173-01p

In response to a notice published in the *Federal Register* on March 6, 2000 (65 FR 11758-11759, Docket no. 99-036-1), APHIS received 10 comments on the environmental assessment (EA) prepared for extension request number 99-173-01p during the designated 30-day comment period which ended April 5, 2000. These comments were received from State potato commissions, a potato growers association, an organic consumers association, the USDA's Agricultural Research Service, a State university, a State university agricultural experiment station, plant virologists, a farmer, and a private individual. Six of the comments were in favor of the extension request, and four were in opposition. Two comments received after the close of the comment period from private individuals also expressed opposition to the deregulation of potato line RBMT22-82.

The majority of commenters supporting the extension request stressed the subject potato line's effectiveness in resisting damage from the Colorado potato beetle (CPB) and potato leaf roll virus (PLRV) and an associated dramatic decrease in the use of pesticides. An entomologist in support of deregulation for potato line RBMT22-82 reported beneficial impacts on populations of nontarget organisms observed during field trials of this potato line when compared with varieties requiring the use of pesticides to control CPB and PLRV. Similar positive environmental effects have been reported in the recently published study prepared by the National Research Council (NRC), *Genetically Modified Pest-Protected Plants: Science and Regulation* (National Academy Press, April 2000, pp. 110-111).

A number of the commenters writing in opposition to the extension request stressed the need for additional research and testing of genetically engineered crop plants to resolve questions concerning gene flow, the development of insect resistance, effects on nontarget organisms, and the creation of new viruses. APHIS addressed these issues in both the EA prepared for petition number 97-204-01p and in the EA prepared for the subject extension request. In brief, we noted that because potato line RBMT22-82 is of the male sterile Russet Burbank variety, the production of viable pollen is unlikely and thus the possibility of gene flow to nonengineered potatoes is virtually eliminated. The development of insect (CPB) resistance has been recognized by the U.S. Environmental Protection Agency (EPA) as an issue, and a resistance management strategy has been adopted by Monsanto and EPA. For an assessment of insect resistance management techniques, please refer to the above-mentioned NRC study at pages 96 through 99. Further, EPA has determined that potatoes containing the plant pesticide Cry3A will not harm nontarget and beneficial insects or endangered and threatened species, and APHIS' procedures for addressing the impacts of potato line RBMT22-82 on endangered and threatened species have been found adequate in our discussions with the U.S. Fish and Wildlife Service.

One comment discussed the absence of data and information on which to base an understanding of plant viruses and concluded that virus-containing plants such as RBMT22-82 were not suitable for release into the environment because of the potential for the development of

USDA/APHIS Decision on Monsanto Request (99-173-01p) Seeking an Extension of Determination of Nonregulated Status for PLRV and CPB resistant Potato Line RBMT22-82

Environmental Assessment

March 2000

Animal and Plant Health Inspection Service
U.S. Department of Agriculture
4700 River Road, Unit 147
Riverdale, MD 20737–1237

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I. THE REGULATED ARTICLE

The Animal and Plant Health Inspection Service (APHIS), U.S. Department of Agriculture (USDA), has prepared an Environmental Assessment (EA) in response to a request (APHIS number 99-173-01p) from Monsanto Company (Monsanto) for an extension of a determination of nonregulated status issued for potato leaf roll virus (PLRV) and Colorado potato beetle (CPB) resistant Russet Burbank potato transformation event RBMT21-129 (the antecedent organism in APHIS number 97-204-01p). The Monsanto extension request claims that a new Russet Burbank potato line, RBMT22-82, does not present a plant pest risk, and should therefore no longer be a regulated article under regulations at 7 CFR Part 340, based on its similarity to the antecedent organism.

Potato line RBMT22-82 has been developed as a means of providing season-long control of the two damaging pests of potato crops, Colorado potato beetle, *Leptinotarsa decemlineata* (Say), and the potato leaf roll virus. The genes conferring resistance to CPB and PLRV were introduced via genetic engineering techniques. These techniques enabled the developer to express in the potato plants: (a) the gene *cry3A* from the soil bacterium *Bacillus thuringiensis* subsp. *tenebrionis* encoding a highly selective insecticidal delta-endotoxin crystalline protein, Cry3A, (b) the open reading frame (*orf*) 1 and 2 gene from PLRV that encodes a helicase and replicase, and (c) the selectable marker gene encoding the enzyme, 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) gene, isolated from *Agrobacterium* strain CP4. CP4 EPSPS encodes an enzyme which is naturally tolerant to glyphosate, the active ingredient of Roundup® herbicide, that is used in the selection of transformed cells. This gene is used solely as a marker gene and during commercial use of these plants, glyphosate will not be applied to these plants. All the genes were introduced via a well-characterized procedure that results in direct introduction of genes into plant genomes.

Monsanto submitted its extension request after the completion of field tests of potato leaf roll virus and Colorado potato beetle resistant potatoes conducted under APHIS permit and notification numbers: 93-362-01r, 94-217-02r, 94-342-01r, 96-277-01r, 97-017-03r, 98-068-01n, 98-068-08n, 98-068-09n, 98-068-10n, 98-121-08n, and 98-132-09n. The applicant reported no deleterious effects on plants, nontarget organisms, threatened and endangered species, or the environment from any of these field tests. Field tests in the United States were performed under conditions of physical and reproductive confinement.

Regulatory status of event RBMT22-82 at the Food and Drug Administration (FDA) and the Environmental Protection Agency (EPA). FDA completed its review of these potatoes in 1998 as described in the FDA's Statement of Policy: Foods Derived From New Plant Varieties (available at 57 FR 22984, May 29, 1992 or electronically at http://vm.cfsan.fda.gov/~lrd/biotechm.html). EPA has granted a

tolerance exemption for the two pesticidal genes for all potatoes (see appendices A and B).

The development of resistance of the CPB to Cry3A protein is recognized as an scientific and environmental issue. A voluntary resistance management strategy has been adopted by Monsanto and EPA with input from USDA to delay the development of resistant CPB and to extend the period of time that this pesticide is effective against CPB (http://www.epa.gov/fedrgstr/EPA-PEST/1995/May/Day-03/pr-243.html or 60 FR 21725, May 3, 1995, and the "Pesticide Fact Sheet for Plant-Pesticide Bacillus thuringiensis Cry3(A) Delta Endotoxin and the Genetic Material Necessary for Its Production in Potato", Conditional Registration that is available upon request).

II. THE ANTECEDENT ORGANISM

The antecedent organism line, RBMT21-129, contained the following sequences:

> The *nptII* gene, encoding the enzyme neomycin phosphotransferase, whose transcription is directed by the nopaline synthase promoter (Fraley et al., 1983) and whose termination sequences are also derived from nopaline synthase gene (Depicker et al., 1982; Bevan et al., 1983). Both regulatory sequences are from *A. tumefaciens.*
>
> The PLRV *orf* 1 and 2 and associated intergenic region whose transcription is directed by the figwort mosaic 35S promoter (Richins et al., 1987) coupled with the soybean heat shock protein leader sequence (Rascke et al., 1988) and whose termination sequences are derived from the pea ribulose-1,5-bisphosphate carboxylase small subunit gene (Coruzzi et al., 1984).
>
> The *cry3A* gene whose transcription is directed by the *Arabidopsis thaliana* ribulose-1,5- bisphosphate carboxylase small subunit gene (Almedia et al., 1989; Wong et al., 1992) and whose termination sequences are derived from nopaline synthase (Depicker et al., 1982; Bevan et al., 1983).

Also, the left and right border sequences from *A. tumefaciens* are inserted into the potato chromosome.

III. SIMILARITIES AND DIFFERENCES BETWEEN ANTECEDENT ORGANISM AND RBMT22-82

Data provided by Monsanto and analyzed by APHIS demonstrated that line RBMT22-82 contains the following plant expressed sequences:

> The PLRV *orf* 1 and 2 and associated intergenic region whose transcription is directed by the figwort mosaic 35S promoter (Richins et al., 1987) coupled with the soybean heat shock protein leader sequence (Rascke et al., 1988) and whose

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

The *cry3A* gene whose transcription is directed by the *Arabidopsis thaliana* ribulose-1,5- bisphosphate carboxylase small subunit gene (Almedia et al., 1989; Wong et al., 1992) and whose termination sequences are derived from nopaline synthase (Depicker et al., 1982; Bevan et al., 1983).

The 5-enolpyruvylshikimate-3-phosphate synthase gene from *Agrobacterium* sp. strain CP4 whose transcription is directed by the nopaline synthase promoter from the Ti plasmid from *A. tumefaciens* (Fraley *et al.* 1983), a chloroplast transit peptide sequence from *Arabidopsis thaliana* EPSPS gene (Klee *et al.* 1987); and whose termination sequences are derived from the pea ribulose-1,5-bisphosphate carboxylase small subunit gene.

Also present but not expressed in the plant are the left and right border sequences from *A. tumefaciens*, the bacterial origin of replication *ori-322* from *E. coli*, and the adenyltransferase gene (*aad*) from transposon TN7 from *E. coli* conferring spectinomycin/streptomycin resistance under bacterial promoter. Monsanto submitted data that demonstrated that adenyltransferase enzyme was not produced in the transgenic plant.

There are several minor differences between RBMT22-82 and the antecedent organism. The antecedent organism had the *nptII* gene that confers resistance to antibiotics kanamycin and neomycin used in the selection of transformed cells, while RBMT22-82 has the CP4 EPSPS gene. RBMT22-82 also has the *ori-322* and the *aad* gene sequences present in its genome. However, they do not result in any protein being produced in the plant.

Line RBMT22-82 is PLRV and CPB resistant based on the data submitted by the applicant and analyzed by APHIS. APHIS concludes this transgenic plant exhibits the agronomic characteristics essentially identical to the antecedent organism RBMT21-129.

IV. POTENTIAL ENVIRONMENTAL IMPACTS

This EA is tiered to the original EA of 97-204-01p (see Appendix A) in which potential for impacts to the human environment through unrestricted use in agriculture of the antecedent organism have been addressed in detail. Addressed below are issues that have been raised since the previous EA was prepared.

Impacts of the use of the antecedent organism on insecticide use. The antecedent organism has not been used commercially on a large scale. The July 1999 issue of *Potato Grower* reported that use of the antecedent organism on 5,000 acres (out of total potato acreage of more than one million) reduced insecticide inputs by farmers. If

these transgenic plants are adopted for use by farmers, then data would be available to assess if use of genetic-based resistance to these pests will result in a reduction of chemical insecticides. Changes in pesticide use associated with transgenic plants is being monitored by USDA's Economic Research Service. Preliminary data for other crops that have been used on a larger scale are available at http://www.econ.ag.gov/whatsnew/issues/gmo/index.htm. The preliminary data indicates that under certain conditions (e.g., high insect pressures) use of Bt cotton or Bt corn has resulted in a reduction of chemical pesticides.

Potential impacts of Line RBMT22-82 on children, minorities, and organic farmers.

Following the directive specified in Executive Order 13045 to identify and assess environmental health or safety risks that might disproportionately affect children (to the extent permitted by law and appropriate and consistent with the agency's mission), we report that no toxicity or allergenicity is known for the three genes or their gene products. Bernstein *et al.* (1999) reported immune response in farm workers after exposure to commercial formulations of powdered *Bacillus thuringiensis*. These commercial formulations may contain multiple copies of single toxin genes and a variety of different-sized toxin genes (Thorne et al. 1986), or various less well defined heat-labile toxins that may be responsible for the toxicity of some isolates of *B. thuringiensis* to non-target organisms including mice, some aquatic insects, and fish (Beegle and Yamamoto, 1992). Another recently-analyzed toxin class from *Bacillus* isolates is Vip3A (Estruch et al., 1996), which was shown to function in a similar manner as the delta-endotoxins (Yu et al., 1997). The dried bacteria and spores from the production of the commercial grade of biopesticide also contains many potential allergens. However, the data using cloned single toxin genes that lack the above contaminants support the conclusion that Cry3A does not have characteristics of allergens. Under simulated gastric (acidic) conditions, CP4 EPSPS and Cry3A proteins are rapidly degraded (OECD, 1999). They are not likely to be glycosylated, and have no sequence homologies with other allergenic proteins; thus, the likelihood of allergenicity is low. APHIS believes that proteins that have no significant amino acid homology to known mammalian protein toxins, that are readily inactivated by heat or mild acidic conditions, and that are readily degraded in an in vitro digestibility assay, would have little likelihood for displaying oral toxicity. When proteins are toxic, they are known to act via acute mechanisms and at very low dose levels (Sjoblad *et al.*, 1992). Children are not likely to consume significantly more of the engineered product than are adults. With respect to *orf* 1 and 2, no protein is produced from PLRV *orf* 1 and 2 in the transgenic plant. This protein is produced in PLRV-infected potatoes and as such is a part of the human diet. There is no reports that this protein has any allergenic properties. Therefore, our evaluation reveals no potential for impact to the health or safety of children or adults of line RBMT22-82.

Under Executive Order 12898, APHIS is required to state any possible adverse impacts upon minorities. APHIS can envision no negative impact to minorities from

consuming these potatoes, or from handling them during processing, planting, or harvesting. If this genetic-based resistance is accepted by farmers, and the chemical pesticides that are currently used to control these pests are eliminated or reduced, this should reduce exposure of all farmer workers to chemicals. As compared to the common pesticides used to control these pests, the biopesticides Cry3A and *orf* 1 and 2 in RBMT22-82 have significantly fewer potential hazards to humans (see Appendix C).

Organic farmers should not be impacted by the expected commercial use of this product since: (a) nontransgenic Russet Burbank seed potatoes will likely still be sold; (b) the engineered potatoes will be advertised as PLRV and CPB resistant to easily distinguish them from the susceptible Russet Burbank; and (c) gene flow from engineered to nonengineered potatoes is not an issue since Russet Burbank is male sterile.

Potential Impact on Nontarget Organisms, Including Beneficial Organisms.

Since APHIS' approval of the original petition, there are no reports or data that suggest that the use of RBMT21-129 has had any impact on nontarget organisms or threatened or endangered species. On July 28, 1999, APHIS met with Fish and Wildlife Service and they determined our procedures to be adequate for addressing the impact of line RBMT22-82 on threatened and endangered species. Therefore, APHIS concludes that there is no reason to believe that deleterious effects or significant impacts on nontarget organisms including threatened and endangered species or beneficial organisms, would result from the expression of PLRV orf 1 and 2 or Cry3A in RBMT22-82 (see appendices A and B).

There is no reason to believe that deleterious effects or significant impacts on nontarget organisms, including threatened and endangered species or beneficial organisms, would result from the CP4 EPSPS conferring glyphosate tolerance that was used as a selectable marker during laboratory and field development of Monsanto transgenic potato transformation events. Data supports that this protein is not allergenic nor a toxin (see above). The application of glyphosate to this potato line when grown on commercial scale is unlikely and would require additional review by the Environmental Protection Agency.

Because the regulated article RBMT22-82 is agronomically similar to the antecedent organism RBMT21-129, it does not present any new potential environmental impact issues other than addressed in the EA associated with determination on petition number 97-204-01p.

V. CONCLUSIONS

APHIS has evaluated available information from the scientific literature and scientific community as well as data submitted by Monsanto that characterized CPB- and

PLRV-resistant potatoes. APHIS has considered the foreseeable consequences of removing CPB- and PLRV-resistant potato line RBMT22-82 from these regulations, and has reached the following conclusions:

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

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

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

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

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

VI. REFERENCES

See appendix A and B for citations in this EA that are not listed below.

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Sjoblad, R.D., J.T. McClintock, and R. Engler. 1992. Toxicological considerations for protein components of biological pesticide products. Reg. Tox. Pharmacol. 15:3-9.

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Yu, C., M.A. Mullins, G.W. Warren, M.G. Koziel, and Juan J. Estruch. 1997. The *Bacillus thuringiensis* vegetative insecticidal protein Vip3A lyses midgut epithelium cells of susceptible insects. Appl. Environ. Microbiol. 63:532-536.

VII. REVIEWERS

Permits and Risk Assessment

John H. Payne, Ph.D, Assistant Director
David S. Heron, Ph.D., Biotechnologist (Reviewer)
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Michael A. Lidsky, J.D., LL.M., Assistant Director
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VIII. AGENCY CONTACT

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Appendix A: Environmental Assessment and Finding of No Significant Impact 97-204-01p

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

Environmental Assessment and
Finding of No Significant Impact

December 1998

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

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

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I. SUMMARY

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

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

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

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

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

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

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

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

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

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

II. BACKGROUND

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

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

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

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

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

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

B. APHIS Regulatory Authority.

APHIS regulations at 7 CFR Part 340, which were promulgated pursuant to authority granted by the Federal Plant Pest Act, as amended (7 U.S.C. 150aa-150jj) and the Plant Quarantine Act, as amended (7 U.S.C. 151-164a, 166-167) regulate the introduction (importation, interstate movement, or release into the environment) of certain genetically engineered organisms and products. An organism is no longer subject to the regulatory requirements of 7 CFR Part 340 when it is demonstrated not to present a plant pest risk. A genetically engineered organism is considered a regulated article if the donor organism, recipient organism, vector or vector agent used in engineering the organism belongs to one of the taxa listed in the regulation and is also a plant pest, or if there is reason to believe that it is a plant pest. CPB- and PLRV-resistant potatoes described in the Monsanto petition have been considered regulated articles because they contain the *orf* 1 and 2 gene from PLRV, noncoding DNA regulatory sequences derived from plant pathogens, and the vector agent used to deliver the plasmid vector is a plant pathogen.

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

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

C. EPA and FDA Regulatory Authority

These genetically engineered potato lines are also currently subject to regulation by other agencies. The EPA is responsible for the regulation of pesticides under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), as amended (7 U.S.C. 136 *et seq.*). FIFRA requires that all pesticides, including insecticides, be registered prior to distribution or sale, unless exempt by EPA regulation. Under the Federal Food, Drug, and Cosmetic Act (FFDCA), as amended (21 U.S.C. 301 *et seq.*), pesticides added to (or contained in) raw agricultural commodities generally are considered to be unsafe unless a tolerance or exemption from tolerance has been established. Residue tolerances for pesticides are established by EPA under the FFDCA, and the FDA enforces the tolerances set by the EPA.

The FDA policy statement concerning regulation of products derived from new plant varieties, including those genetically engineered, was published in the Federal Register on May 29, 1992, and appears at 57 FR 22984-23005. Safety concerns for human and animal consumption of products with kanamycin resistance are also specifically addressed by the FDA in 21 CFR Parts 173 and 573.

III. PURPOSE AND NEED

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

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

IV. ALTERNATIVES

A. No Action.

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

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

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

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

V. AFFECTED ENVIRONMENT AND POTENTIAL ENVIRONMENTAL IMPACTS

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

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

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

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

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

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

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

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

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

1) Potential for gene introgression into other potato cultivars.

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

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

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

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

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

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

C. Development of Viral Symptoms on Some Transgenic Plants

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

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

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

D. Potential for the Appearance of New Plant Viruses

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

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

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

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

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

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

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

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

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

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

VI. CONCLUSION

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

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

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

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

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

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

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

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

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Appendix B: Determination of Nonregulated Status 97-204-01p

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

Prepared by
United States Department of Agriculture
Animal and Plant Health Inspection Service
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I. SUMMARY

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

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

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

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

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

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

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

II. BACKGROUND

A. APHIS regulatory authority.

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

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

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

B. EPA and FDA regulatory authority

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

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

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

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

III. RESPONSE TO COMMENTS

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Development of Viral Symptoms on Some Transgenic Plants

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

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

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

A. Recombination

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

B. Transcapsidation

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

C. Synergy

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

D. Satellites and host RNA polymerases

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

V. CONCLUSION

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

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Appendix C: A comparison of environmental and human health effects of common pesticides used to control aphids and Colorado potato beetle in potatoes.

	CryIIIA	Imidacloprid (Admire®)	Esfenvalerate (Asana®)	Spinosad (SpinTor®)	Endosulfan (Thiodan®)
Environmental Fate & Nontarget soil organisms	Because of literature reports describing adverse effects on soil invertebrates from conventional B. thuringiensis products and the potential for exposure from B.t. CryIII(A) delta endotoxin protein in the plant debris left in the field after harvest that soil organisms will feed upon, these studies will need to be submitted by the registrant. 200 ppm Cry3A microbial-produced toxin showed no effect on *Folsomia candida* and *Xenylla grisea*	The half-life of imidacloprid in soil is 48-190 days, depending on the amount of ground cover. Organic material aging may also affect the breakdown rate of imidacloprid. There is generally not a high risk of groundwater contamination with imidacloprid. The chemical is moderately soluble, and has moderate binding affinity to organic materials in soils. There is potential for the compound to move through porous soil types depending on irrigation practices . Breakdown of Chemical in Surface Water: Half-life in water is much >31days at pH 5, 7 and 9. Breakdown in vegetation: Imidacloprid penetrates the plant, and moves from the stem to the tips of the plant. Metabolism in a variety of crop and application types has been characterized.	Under field conditions, esfenvalerate is moderately persistent with a half-life ranging from about 15 days to three months depending on soil type . In a soil laboratory study, 17% of the applied chemical was lost in 90 days. Esfenvalerate and its breakdown products are relatively immobile in soil and thus pose little risk to groundwater . The compounds ability to bind to soil increases with increasing organic matter. It is very insoluble in water. Fenvalerate has not been found in over 100 tested groundwater supplies . Breakdown in water: Esfenvalerate will break down in water to one-half of the original amount (half-life) in about twenty-one days due to sunlight . Breakdown in vegetation: The parent compound is the residue most often found on foliage. In a series of trials in Canada where 16 varieties of fruits and vegetables were grown under typical outdoor conditions, no degradation products or metabolites were found (tests were sensitive to 0.05 mg/kg). Sampling was from 1 to 112 days after application. The half-life of esfenvalerate on plant surfaces is 2 to 4 weeks.	Spinosad is relatively short-lived in the field and photodegrades rapidly, half- lives less than one day. Leaching data show that Spinosad and its aged residues are unlikely to leach in most soils, are relatively immobile and poses little threat to groundwater. -Photodegradation in water: Half lives in summer sunlight and pH 7 were 0.93 day for spinosyn A and 0.82 day for spinosyn D. -Photodegradation in soil: Half lives on Commerce silt loam soil exposed to sunlight for up to 30 days were 82 days for spinosyn A and 44 days for spinosyn D. -Aerobic soil metabolism: Half lives were 9.4 to 17.3 days for spinosyn A and 14.5 days for spinosyn D. -Anaerobic aquatic metabolism: Half lives were 161 days for spinosyn A and 250 days for spinosyn D.	Endosulfan is moderately persistent in the soil environment with a reported average field half-life of 50 days . The two isomers have different degradation times in soil. The half-life for the alpha -isomer is 35 days, and is 150 days for the beta-isomer under neutral conditions. These two isomers will persist longer under more acidic conditions. The compound is broken down in soil by fungi and bacteria . Endosulfan does not easily dissolve in water, and has a very low solubility . It has a moderate capacity to adhere or adsorb to soils . Transport of this pesticide is most likely to occur if endosulfan is adsorbed to soil particles in surface runoff. It is not likely to be very mobile or to pose a threat to groundwater. It has, however, been detected in California well water . Breakdown in water: In raw river water at room temperature and exposed to light, both isomers disappeared in 4 weeks . A breakdown product first appeared within the first week. The breakdown in water is faster (5 weeks) under neutral conditions than at more acidic conditions or basic conditions (5 months) . Under strongly alkaline conditions the half-life of the compound is 1 day. Large amounts of endosulfan can be found in surface water near areas of application . It has also been found in surface water throughout the country at very low concentrations . Breakdown in vegetation: In plants, endosulfan is rapidly broken down to the corresponding sulfate . On most fruits and vegetables, 50% of the parent residue is lost within 3 to 7 days . Endosulfan and its breakdown products have been detected in vegetables

	CryIIIA	Imidacloprid (Admire®)	Esfenvalerate (Asana®)	Spinosad (SpinTor®)	Endosulfan (Thiodan®)
Avian data	The studies were both scientifically sound and no treatment mortality, differences in food consumption or behavior was observed between the dosed (50,000 ppm from potato tubers) and control birds.	Imidacloprid is **toxic** to upland game birds. The LD50 is 152 mg/kg for bobwhite quail, and 31 mg/kg in Japanese quail . Studies with red-winged blackbirds and brown-headed cowbirds concluded that the risk of dietary exposure to birds via treated seeds was minimal because imidacloprid had feeding deterrent or repellent activity .	Esfenvalerate is slightly **toxic** to birds. Oral LD50 values for the compound are 1312 mg/kg in bobwhite quail and greater than 2250 mg/kg in mallard ducks	-Acute oral LD50 northern bobwhite quail and mallard duck: >1333 mg/kg. Slightly **toxic**. -Acute dietary LC50 northern bobwhite quail and mallard duck: >5156 ppm. Practically **nontoxic**.	Endosulfan is highly to moderately **toxic** to bird species, with reported oral LD50 values in mallards ranging from 31 to 243 mg/kg , and in pheasants ranging from 80 to greater than 320 mg/kg . The reported 5-day dietary LC50 is 2906 ppm in Japanese quail. Male mallards from 3 to 4 months old exhibited wings crossed high over their back, tremors, falling, and other symptoms as soon as 10 minutes after an acute, oral dose. The symptoms persisted for up to a month in a few animals
Aquatic Data	Since the B.t.t. insect control protein is contained within the potato tissue, exposure to aquatic organisms is considered to be unlikely.	The toxicity of imidacloprid to fish is **moderately low**. The 96-hour LC50 is 211 mg/l for rainbow trout, 280 mg/l for carp, and 237 mg/l for golden orfe. In tests with the aquatic invertebrate Daphnia, the 48-hour EC50 (effective concentration to cause toxicity in 50% of the test organisms) was 85 mg/l (1). Products that contain imidacloprid may be **very toxic** to aquatic invertebrates.	Based on laboratory studies, fish are **very sensitive** to esfenvalerate. It has a 96-hour LC50 of 0.0003 mg/L in bluegill, 0.0003 mg/L in rainbow trout, 0.001 mg/L in carp, and 0.0002 mg/L in killfish]. The LC50 in Daphnia magna, an aquatic invertebrate, is 0.001 mg/L. The pesticide is very highly **toxic** to these species. Water turbidity, such as would be found in the field, tends to reduce the toxicity of this compound . Bioaccumulation factors in rainbow trout are about 400 times the background (ambient water concentration of the pesticide) levels	-Acute 96-hour LC50 rainbow trout: 30 ppm. Slightly **toxic**. - Acute 96-hour LC50 bluegill sunfish: 5.94 ppm. Moderately **toxic**. - Freshwater fish early life-stage rainbow trout: NOEC 0.498 ppm, LOEC 0.962 ppm, and MATC 0.692 ppm. -Acute 48-hour ECSO daphnid: 14 ppm. Slightly **toxic**. -Freshwater aquatic invertebrate life-cycle daphnid: NOEC 0.0006 ppm, LOIC 0.0012 ppm, MATC 0.0008 ppm. -Acute estuarine 96-hour LC50 sheepshead minnow: 7.9 ppm. Moderately toxic. -Acute estuarine 96-hour LC50 grass shrimp: >9.76 ppm. Moderately toxic. -Acute estuarine 96-hour EC50 eastern oyster: 0.3 ppm. Very highly **toxic**.	Endosulfan is very highly **toxic** to four fish species and both of the aquatic invertebrates studied; in fish species, the reported 96-hour LC50 values were (in ug/L): rainbow trout, 1.5; fathead minnow, 1.4; channel catfish, 1.5; and bluegill sunfish, 1.2. In two aquatic invertebrates, scuds (G. lacustris) and stoneflies (Pteronarcys), the reported 96-hour LC50 values were, respectively, 5.8 ug/L and 3.3 ug/L. The bioaccumulation for the compound may be significant; in the mussel (Mytelus edulis) the compound accumulated to 600 times the ambient water concentration .

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Mammalian toxicity	Approximately 176 different B. thuringiensis products have been registered since 1961 by the EPA has not received any reports of dietary toxicity attributable to their use. This is not unexpected since there are no known equivalent receptor sites in mammalian species which could bind the toxin.	Imidacloprid is a General Use Pesticide, and is classified by EPA as both a toxicity class II and class III agent, and must be labeled with the signal word "Warning" or "Caution". Acute Toxicity: Imidacloprid is moderately toxic. The (LD50) of the oral dose of technical grade imidacloprid is 450 mg/kg body weight in rats, and 131 mg/kg in mice. The 24-hour dermal LD50 in rats is >5,000 mg/kg. It is considered non-irritating to eyes and skin (rabbits), and non-sensitizing to skin (guinea pigs) . In acute inhalation toxicity tests with rats, the airborne LC50 is > 69 mg/meters cubed air in the form of an aerosol, and >5323 mg/meters cubed air in the form of dust. These values represent the maximum attainable airborne concentrations .	Acute toxicity: Esfenvalerate is a moderately toxic compound via the oral route. The reported oral LD50 of esfenvalerate is 458 mg/kg in rats. It is slightly toxic via the dermal route, with a reported dermal LD50 of 2500 mg/kg in rabbits. It is practically non-toxic via inhalation, with a reported inhalation LC50 of greater than 2.93 mg/L in rats. Because esfenvalerate is a relatively new compound it has little usage history. The bulk of evidence related to acute poisonings in humans due to esfenvalerate comes from incidents in India. Nearly 600 individual cases of poisoning were reported between 1982 and 1988. These cases were due to improper handling of the pesticide. Acute toxic effects were observed in workers and among the general public. Symptoms of acute poisoning included dizziness, burning and itching (which was worsened by sweating and washing). Severe cases of direct contact caused blurred vision, tightness in the chest, and convulsions (2). The changes appear to be reversible. In rats, high acute exposure to esfenvalerate produced muscle incoordination, tremors, convulsions, nerve damage, and weight loss. The compound may produce nausea, vomiting, headache, temporary nervous system effects such as weakness, tremors, and incoordination at acute exposure levels in humans. Esfenvalerate is a strong eye irritant, producing tearing or blurring of vision. Chronic toxicity: Rats fed fenvalerate at concentrations of approximately 12.5 mg/kg/day for two years had no compound-related changes in the blood or urine [12]. In other studies significant reduction in body weight was the main adverse effect seen in both rats and mice of	With respect to subchronic toxicity spinosad was evaluated in 13-week dietary studies and showed NOEL's of 4.9 mg/kg/day in dogs, 6 mg/kg/day in mice, and 8.6 mg/kg/day in cats. No dermal toxicity or systemic toxicity occurred in a 21-day repeated dose dermal toxicity study in rabbits given 1000 mg/kg/day (limit dose). Based on chronic toxicity testing with spinosad in the dog, the most sensitive species tested, a RfD of 0.0268 mg/kg/day is being established based on a NOEL of 2.68 mg/kg/day and an uncertainty factor of 100. There was no evidence of carcinogenicity in two rodent species at all dosages tested. Mutagenicity studies showed no mutagenic activity associated with spinosad. There was no developmental effects observed in two oral developmental toxicity studies in rats and rabbits up to the highest dose tested (HDT) . The NOEL found for maternal and pup effects was 10 mg/kg/day . (HDT) . Neonatal effects at 100 mg/kg/day were attributed to maternal toxicity. Spinosad did not cause neurotoxicity in rats in acute, subchronic or chronic toxicity studies.	**Acute toxicity**: Endosulfan is highly toxic via the oral route, with reported oral LD50 values ranging from 18 to 160 mg/kg in rats, 7.36 mg/kg in mice, and 77 mg/kg in dogs ,9 . It is also highly toxic via the dermal route, with reported dermal LD50 values in rats ranging from 78 to 359 mg/kg . Endosulfan may be only slightly toxic via inhalation, with a reported inhalation LC50 of 21 mg/L for 1 hour, and 8.0 mg/L for 4 hours . It is reported not to cause skin or eye irritation in animals . The alpha-isomer is considered to be more toxic than the beta-isomer . Animal data indicate that toxicity may also be influenced by species and by level of protein in the diet; rats which have been deprived of protein are nearly twice as susceptible to the toxic effects of endosulfan . Solvents and/or emulsifiers used with endosulfan in formulated products may influence its absorption into the system via all routes; technical endosulfan is slowly and incompletely absorbed into the body whereas absorption is more rapid in the presence of alcohols, oils, and emulsifiers . Stimulation of the central nervous system is the major characteristic of endosulfan poisoning . Symptoms noted in acutely exposed humans include those common to the other cyclodienes, e.g., incoordination, imbalance, difficulty breathing, gagging, vomiting, diarrhea, agitation, convulsions, and loss of consciousness . Reversible blindness has been documented for cows that grazed in a field sprayed with the compound. The animals completely recovered after a month following the exposure . In an accidental exposure, sheep and

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Mammalian toxicity continued		24 mg/kg/day based on decreased body weight and skeletal abnormalities observed at 72 mg/kg/day (highest dose tested) . Mutagenic Effects: Imidacloprid may be weakly mutagenic. In a battery of 23 laboratory mutagenicity assays, imidacloprid tested negative for mutagenic effects in all but two of the assays. It did test positive for causing changes in chromosomes in human lymphocytes, as well as testing positive for genotoxicity in Chinese hamster ovary cells. Carcinogenic Effects: Imidacloprid is considered to be of minimal carcinogenic risk, and is categorized by EPA as a "Group E" carcinogen (evidence of noncarcinogenicity for humans). Organ Toxicity: In short-term feeding studies in rats, only with very high doses .			
EPA Toxicity class	EPA toxicity class III	Imidacloprid is a General Use Pesticide, and is classified by EPA as both a toxicity class II and class III agent, and must be labeled with the signal word "Warning" or "Caution"	Is a moderately toxic pesticide in EPA toxicity class II; products containing it must contain the Signal Word WARNING on the label	Classified as a toxicity Category III pesticides and are labeled with the signal word "Caution" based on the acute dermal study.	Toxicity class I. Signal words: DANGER POISON depending on formulation
EDF - Integrated Environmental Rankings/ Combined human & ecological scores	Not ranked	Not ranked	Not ranked	Not ranked	50 to 75% on the least to most hazardous scale with 100% being the most hazardous

CryIIIA data from Monsanto's petition 99-173-01p. Data for other pesticides from the following sources:

a. EXTOXNET Pesticide Information Profile at http://ace.ace.orst.edu/info/extoxnet/pips/imidaclo.htm
b. The Environmental Defense Fund's Chemical Scorecard at http://www.scorecard.org/chemical-profiles/
c. Pesticide Fact Sheet on Spinosad 1997 at http://www.epa.gov/opprd001/factsheets/spinosad.htm
d. Imidacloprid; Pesticide Tolerances at http://www.epa.gov/fedrgstr/EPA-PEST/1998/September/Day-18/p25085.htm
e. British Crop Protection Council, The BioPesticide Manual, 1999.
f. British Crop Protection Council, The Pesticide Manual, 1999.
g.. L F Adams, C-L Liu, S C MacIntosh and R L Starnes. 1996. Diversity and biological activity of *Bacillus thuringiensis*. In *Crop Protection Agents from Nature: Natural Products and Analogues*, 360-88, Royal Society of Chemistry, Cambridge, UK.
h. McClintock, J.T., C.R. Schaffer, and R.D. Sjoblad. 1995b. A comparative review of the mammalian toxicity of *Bacillus thuringiensis*-based pesticides. Pestic. Sci. 45:95-105.
i. Sjoblad, R.D., J.T. McClintock, and R. Engler. 1992. Toxicological considerations for protein components of biological pesticide products. Reg. Tox. Pharmacol. 15:3-9.