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

DEPARTMENT OF AGRICULTURE

Animal and Plant Health Inspection Service

[Docket No. 95-041-2]

Availability of Determination of Nonregulated Status for Genetically Engineered Corn

AGENCY: Animal and Plant Health Inspection Service, USDA.

ACTION: Notice.

SUMMARY: We are advising the public of our determination that the Monsanto Company's corn line designated as MON 80100 that has been genetically engineered for insect resistance is no longer considered a regulated article under our regulations governing the introduction of certain genetically engineered organisms. Our determination is based on our evaluation of data submitted by the Monsanto Company in its petition for a determination of nonregulated status, an analysis of other scientific data, and our review of comments received from the public in response to a previous notice announcing our receipt of the Monsanto Company petition. This notice also announces the availability of our written determination document and its associated environmental assessment and finding of no significant impact.

EFFECTIVE DATE: August 22, 1995.

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

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

SUPPLEMENTARY INFORMATION:

Background

On April 3, 1995, the Animal and Plant Health Inspection Service (APHIS) received a petition (APHIS Petition No. 95-093-01p) from the Monsanto Company (Monsanto) of St. Louis, MO, seeking a determination that corn designated as MON 80100 that has been genetically engineered for insect resistance does not present a plant pest risk and, therefore, is not a regulated article under APHIS' regulations in 7 CFR part 340.

On June 7, 1995, APHIS published a notice in the **Federal Register** (60 FR 30061-30062, Docket No. 95-041-1) announcing that the Monsanto petition had been received and was available for public review. The notice also discussed the role of APHIS, the Environmental Protection Agency (EPA), and the Food and Drug Administration in regulating the subject corn line and food products derived from it. In the notice, APHIS solicited written comments from the public as to whether corn line MON 80100 posed a plant pest risk. The comments were to have been received by APHIS on or before August 7, 1995.

APHIS received nine comments on the Monsanto petition, from farmers, industry, universities, a growers association, and a State department of agriculture. All the commenters expressed support for the subject petition.

Analysis

Monsanto's corn line MON 80100 has been genetically engineered to express a CryCIA(b) insect control protein derived from the common soil bacterium *Bacillus thuringiensis* subsp. *kurstaki* (*Btk*). *Btk* proteins are effective against certain lepidopteran insects, including European corn borer (ECB), a major corn pest. Results of field tests conducted by Monsanto under permits and notifications granted by APHIS and under an experimental use permit obtained from EPA indicate that corn plants producing the CryCIA(b) protein were protected throughout the growing season from leaf and stalk feeding damage caused by ECB. In addition to expressing the CryCIA(b) protein, the plants also express the selectable marker enzyme 5-enolpyruvylshikimate-3-phosphate synthase (CP4 EPSPS). The *cryIA(b)* gene and the CP4 EPSPS marker gene were introduced into the subject corn line by a particle acceleration method and their expression is under the control of the enhanced 35S promoter derived from the plant pathogen cauliflower mosaic virus.

The subject corn line has been considered a regulated article under APHIS' regulations in 7 CFR part 340 because it contains certain gene sequences derived from plant-pathogenic sources. However, evaluation of field data reports from field tests of corn line MON 80100 conducted since 1992 indicates that there were no deleterious effects on plants, nontarget organisms, or the environment as a result of the subject corn plants' release into the environment.

Determination

Based on its analysis of the data submitted by Monsanto and a review of other scientific data, comments received from the public, and field tests of the subject corn, APHIS has determined that corn line MON 80100: (1) Exhibits no plant pathogenic properties; (2) is no more likely to become a weed than lepidopteran-insect-resistant corn developed through traditional breeding techniques; (3) is unlikely to increase the weediness potential of any other cultivated plant or native wild species with which it can interbreed; (4) should not cause damage to raw or processed agricultural commodities; (5) is unlikely to harm organisms beneficial to the agricultural ecosystem; and (6) when cultivated, should not reduce the ability to control insects in corn and other crops. APHIS has also concluded that there is a reasonable certainty that new varieties developed from corn line MON 80100 will not exhibit new plant pest properties, i.e., properties substantially different from any observed in the field tested corn line MON 80100, or those observed in corn in traditional breeding programs.

The effect of this determination is that an insect-resistant corn line designated as MON 80100 is no longer considered a regulated article under APHIS' regulations in 7 CFR part 340. Therefore, the notification requirements pertaining to regulated articles under those regulations no longer apply to the field testing, importation, or interstate movement of corn line MON 80100 or its progeny. However, the importation of the subject corn line or seed capable of propagation is still subject to the restrictions found in APHIS' foreign quarantine notices in 7 CFR part 319.

National Environmental Policy Act

An environmental assessment (EA) has been prepared to examine the potential environmental impacts associated with this determination. The EA was prepared in accordance with: (1) The National Environmental Policy Act of 1969 (NEPA)(42 U.S.C. 4321 *et seq.*), (2) Regulations of the Council on Environmental Quality for Implementing the Procedural Provisions of NEPA (40 CFR parts 1500–1508), (3) USDA Regulations Implementing NEPA (7 CFR part 1b), and (4) APHIS' NEPA Implementing Procedures (7 CFR part 372; 60 FR 6000–6005). Based on that EA, APHIS has reached a finding of no significant impact (FONSI) with regard to its determination that the subject corn line 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 28th day of August 1995.

Terry L. Medley,

Acting Administrator, Animal and Plant Health Inspection Service.

[FR Doc. 95–21847 Filed 9–1–95; 8:45 am]

BILLING CODE 3410–34–P

USDA/APHIS Petition 95-093-01 for Determination of Nonregulated Status for Insect Protected Corn line MON 80100

Environmental Assessment and Finding of No Significant Impact

August 1995

The Animal and Plant Health Inspection Service (APHIS) of the U. S. Department of Agriculture has prepared an environmental assessment in the course of evaluating a petition for determination of nonregulated status for a genetically engineered insect protected corn line called MON 80100. This corn expresses the CryIA(b) protein of *Bacillus thuringiensis* subsp. *kurstaki*. APHIS received a petition from the Monsanto Company regarding the status of the insect protected corn line MON 80100 as a regulated article under APHIS regulations at 7 CFR Part 340. APHIS has conducted an extensive review of the petition, supporting documentation, and 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 insect protected corn line MON 80100 shall no longer be a regulated article.

John H. Payne, Ph.D.
Acting Director
Biotechnology, Biologics, and Environmental Protection
Animal and Plant Health Inspection Service
U.S. Department of Agriculture
Date: AUG 22 1995

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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) before deciding on the regulated status of a genetically engineered line of lepidopteran (caterpillar) insect protected corn designated hereafter as corn line MON 80100. The Monsanto Company petitioned APHIS requesting a determination on the regulated status of corn line MON 80100 that has been a regulated article under APHIS regulations. Interstate movements and field tests of corn line MON 80100 have been conducted under permits issued by or notifications acknowledged by APHIS. Monsanto Company has petitioned APHIS for a determination that corn line MON 80100 does not present a plant pest risk and should therefore no longer be a regulated article under the APHIS regulations found at 7 CFR Part 340.

Corn line MON 80100 has been developed in an effort to protect the corn plants against damage caused by the feeding larvae of European corn borer (*Ostrinia nubilalis* (Hubner)). A gene encoding an insecticidal protein from *Bacillus thuringiensis* subsp. *kurstaki* has been inserted into the corn chromosome. Two selectable genetic markers encoding 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS) and glyphosate oxidoreductase (GOX) were also introduced into the corn chromosome but only to facilitate selection of transformed cells in the laboratory. The genes were introduced by a particle acceleration transformation system that results in direct introduction of genes into plant cell nucleus.

EAs were prepared before granting the permits for field trials of corn line MON 80100. Previous EAs addressed questions pertinent to plant pest risk issues concerning the conduct of field trials under physical and reproductive confinement, but they do not address several issues that are of relevance to the unconfined growth of corn line MON 80100. With respect to these new issues, APHIS concludes the following:

1. Corn line MON 80100 exhibits no plant pathogenic properties. Although pathogenic organisms were used in their development, these corn plants are not infected by these organisms nor can these plants incite disease in other plants.

2. Corn line MON 80100 is no more likely to become a weed than insect-resistant corn developed by traditional breeding or other techniques. Corn is not a serious, principal or common weed pest in the U.S. There is no reason to believe that resistance to insects would enable corn to become a weed pest.

3. Multiple factors act to make gene introgression from corn line MON 80100 into wild or cultivated sexually-compatible plants extremely unlikely. Such rare events should not increase the weediness potential of any resulting progeny or adversely impact biodiversity.

4. Seeds of corn line MON 80100 are substantially equivalent in composition, quality and other characteristics to nontransgenic corn except they contain traces of CryIA(b) protein which should have no adverse impacts on raw or processed agricultural commodities.

5. Corn line MON 80100 poses no harm to threatened or endangered species, exhibits no significant potential either to harm organisms beneficial to agriculture or to impair the ability of farmers to control nontarget insect pests.

6. Cultivation of corn line MON 80100 should not reduce the ability to control insects in corn and other crops.

Therefore, after a review of the available evidence, APHIS concludes that there will be no significant impact on the human environment if corn line MON 80100 were no longer considered a regulated article under regulations at 7 CFR Part 340. APHIS has also concluded that there is a reasonable certainty that new varieties developed from corn line MON 80100 will not exhibit new plant pest properties, i.e., properties substantially different from any observed in the field tested MON 80100 corn line, or those observed in corn in traditional breeding programs.

II. Background

A. Development of Corn Line MON 80100

Monsanto Company has submitted a "Petition for Determination of Non-regulated Status" to the USDA, APHIS for corn plants that contain a gene that protects the corn plants against the feeding damage caused by the larvae of European corn borer. Monsanto Company requested a determination from APHIS that the corn line MON 80100, and any progeny derived from hybrid crosses between this line and other non-transformed corn varieties, no longer be considered regulated articles under 7 CFR Part 340.

European corn borer (ECB) damage to corn plants results in stalk lodging, dropped ears, and damaged grain. Yield reductions due to ECB infestations are estimated to exceed $50 million annually in the State of Illinois alone. *B. thuringiensis* subsp. *kurstaki* produces a family of related toxins (delta-endotoxin) that when ingested by susceptible lepidopteran insects result in their death. These toxins accumulate as crystalline structures in and during the bacterial spore formation. Preparations of *B. thuringiensis* containing delta-endotoxins are used as foliar biopesticides. However, as applied to leaf surfaces, they are not routinely effective against ECB because the insect feeds inside the plants where the foliar biopesticide cannot reach. Monsanto Company has modified the corn plant to produce in green tissues and pollen cells a specific delta-endotoxin, called CryIA(b). During field testing of corn line MON 80100 that express the *cryIA(b) gene*, ECB infestations were significantly reduced as compared to nontransgenic control plants. The *cryIA(b)* gene is expressed from an enhanced 35S promoter (E35S) (Kay *et al*, 1985 and Odell *et al*, 1985) derived from cauliflower mosaic virus, a known plant pest. The *cryIA(b)* gene is joined to nopaline synthase 3' transcription terminator, NOS 3', (Rogers *et al*, 1985). Corn line MON 80100 has also been transformed with two selectable marker genes, the *EPSPS* and *gox* genes. The *EPSPS* and *gox* genes enable the selection of cells in tissue culture that contain the *cryIA(b)* gene. Upon glyphosate treatment, plants or plant cells expressing the EPSPS protein are unaffected since the continued action of the tolerant EPSPS enzyme meets the plant's need for aromatic compounds.

These genes were introduced into corn line MON 80100 via microprojectile bombardment transformation. This is a well-characterized procedure that has been used for nearly a decade for introducing various genes of interest into plant genomes. Southern blot analysis and Mendelian genetics data suggest that the introduced genes are stably integrated into the corn genome and are stably inherited.

Corn line MON 80100 has been field tested since 1992 in the major corn growing regions of the United States under 7 permits and numerous acknowledgements of notifications by APHIS (USDA Permit Nos. 92-232-01, 92-262-01, 93-021-02, 93-021-03, 93-021-04, 93-021-05, 93-021-06 and 64 Notifications during 1994 and 1995). Corn line MON 80100 has been evaluated extensively in laboratory, greenhouse, and field experiments to confirm that it exhibits the desired agronomic characteristics and does not pose a plant pest risk. Although the field tests of corn line MON 80100 have been conducted in agricultural settings, the permit conditions and acknowledgement of notifications for the tests have stipulated physical and reproductive confinement from other plants.

B. APHIS Regulatory Authority

APHIS regulations at 7 CFR Part 340, which were promulgated pursuant to authority granted by the Federal Plant Pest Act, (7 U.S.C. 150aa-150jj) as amended, and the Plant Quarantine Act, (7 U.S.C. 151-164a, 166-167) as amended, regulate the introduction (importation, interstate movement, or release into the environment) of certain genetically engineered organisms and products.
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. Corn line MON 80100, described in the Monsanto Company's petition has been considered a regulated article because noncoding DNA regulatory sequences and portions of the plasmid construct are derived from plant pathogens.

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 APHIS determines that the regulated article is unlikely to pose a greater plant pest risk than the unmodified organism, the Agency can grant the petition in whole or in part. Therefore, APHIS permits under 7 CFR Part 340 would no longer be required for field testing, importation, or interstate movement of that article or its progeny.

C. Environmental Protection Agency (EPA) and Food and Drug Administration (FDA) Regulatory Authority

Corn line MON 80100 is also subject to regulation by other agencies. The EPA is responsible for the regulation of pesticides under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136 *et seq.*). FIFRA requires that all pesticides be registered before distribution or sale, unless exempt by EPA regulation. Accordingly, on January 31, 1995, Monsanto Company submitted an application (EPA File Symbol 524-UIA) to EPA to register this plant-pesticide, i.e., *cry IA(b)* gene and its controlling sequences in corn line MON 80100. The EPA has not yet announced its final decision on this registration application. Before a product may be registered as a pesticide under FIFRA, it must be shown that when used in accordance with widespread and commonly recognized

practice, it will not generally cause unreasonable adverse effects on the environment. On February 2, 1995 Monsanto Company applied for registration of corn line MON 80100 as a pesticide. The EPA has not yet announced its decision on this petition.

Under the Federal Food, Drug, and cosmetic Act (FFDCA) (21 U.S.C. 301 *et seq.*), pesticides added to (or contained in) raw agricultural commodities generally are considered to be unsafe unless a tolerance or exemption from tolerance has been established. Residue tolerances for pesticides are established by EPA under the FFDCA; and the FDA enforces the tolerances set by the EPA. Monsanto Company has submitted to the EPA a pesticide petition (PP 5F4473) proposing to amend 40 CFR part 180 to establish a tolerance exemption for residues of the plant pesticide active ingredient *Btk* protein as expressed in plant cells.

FDA's policy statement concerning regulation of products derived from new plant varieties, including those genetically engineered, was published in the *Federal Register* on May 29, 1992, and appears at 57 **FR** 22984-23005.

III. Purpose and Need

APHIS has prepared this EA before making a determination of the status of corn line MON 80100 as a regulated article under APHIS regulations. The developer of corn line MON 80100, Monsanto Company, submitted a petition to USDA, APHIS requesting that APHIS make a determination that corn line MON 80100 shall no longer be considered a regulated article under 7 CFR Part 340.

This EA was prepared in compliance with the National Environmental Policy Act (NEPA) of 1969 (40 CFR 1500-1508) and the pursuant implementing regulations published by the council on Environmental Quality (42 USC 4331 *et seq.*; 40 CFR 1500-1508; 7 CFR Part 1b; 60 FR 6000-6005).

IV. Alternatives

A. No Action

Under the "no action" alternative, APHIS would not come to a determination that corn line MON 80100 is no longer a regulated article under the regulations at 7 CFR Part 340. Permits from APHIS would still be required for introductions of corn line MON 80100. APHIS would choose this alternative if there were insufficient evidence to demonstrate the lack of plant pest risk from uncontained cultivation of corn line MON 80100.

B. Determination that corn line MON 80100 is no longer a regulated article

Under this alternative, corn line MON 80100 would no longer be a regulated article under the regulations at 7 CFR Part 340. Permits from APHIS would no longer be required for introductions of corn line MON 80100. The basis for such a determination could include a "Finding of No Significant Impact" under the National Environmental Policy Act of 1969 (42 USC 4331 *et seq.*; 40 CFR 1500-1508; 7 CFR Part 1b; 60 FR 6000-6005).

V. Affected Environment and Potential Environmental Impacts

This EA addresses potential environmental impacts from a determination that corn line MON 80100 should no longer be considered a regulated article under APHIS regulations at 7 CFR Part 340. Previous EAs prepared by APHIS with the issuance of permits for field tests of corn line MON 80100 have addressed various attributes of this corn. This EA discusses the genetic modification, and the potential environmental impacts that might be associated with the unconfined cultivation of corn line MON 80100.

Additional technical information is included in the determination document appended to this EA, and incorporated by reference. This includes detailed discussions of the biology of corn, the genetic components used in the construction of corn line MON 80100, and the analyses that lead APHIS to conclude that corn line MON 80100 has no potential to pose plant pest risks.

A. Potential impacts based on increased weediness of corn line MON 80100 relative to traditionally bred insect resistant corn

Although various definitions of the term "weed" have been proposed in the scientific literature, the salient point is that a plant can be considered a weed when it is growing where humans do not want it (Baker 1965; de Wet and Harlan 1975; Muenscher 1980). Baker (1965) lists 12 common attributes that can be used to assess the likelihood that a plant species will behave as a weed. 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 corn is not considered a weed pest and is unlikely to become a weed pest. Corn is a highly inbred, well-characterized crop plant that is not persistent in undisturbed environments without human intervention. Although corn volunteers are not uncommon, they are easily controlled using herbicides or mechanical means. Corn also possess few of the characteristics of plants that are notably successful weeds (e.g., it does not produce abundant, long-lived seed; it does not propagate vegetatively; it does not compete well with other plant species in the environment).

Furthermore, corn has been grown for centuries throughout the world without any reports that it is a serious weed pest. In the United States, corn is not listed as a weed in the major weed references (Crockett 1977; Holm et al. 1979; Muenscher 1980), nor is it present on the lists of noxious weed species distributed by the Federal Government (7 CFR Part 360).

The parent plant of the corn line MON 80100 is an agricultural crop plant that exhibits no appreciable weedy characteristics. The relevant introduced trait, lepidopteran insect resistance, is unlikely to increase weediness of corn line MON 80100. There is no indication that the presence of a *cryIA(b)* gene in resulting corn line MON 80100 will convert it into a weed. In addition, the corn plants have been transformed with two selectable marker genes: *EPSPS*, which confers resistance to the herbicide glyphosate and *gox* which inactivates glyphosate. Eventhough corn is sensitive to glyphosate, it is registerd with EPA for use in corn in a special application manner. The *EPSPS* gene is involved in the biosynthesis of aromatic amino acids. The *gox* gene that encodes the glyphosate metabolizing enzyme glyphosate oxidoreductase (GOX) was cloned from *Achromobacter* sp. strain LBAA (Hallas *et al*, 1988; Barry *et al*, 1992). The enzyme GOX degrades glyphosate by converting glyphosate to aminomethylphosphonic acid (AMPA) and glyoxylate.

These genes do not cause plant disease or damage. Also, their use does not result in the presence of the herbicide in corn and does not imply that glyphosate will be used in the cultivation of the corn. No other attributes of corn line MON 80100 suggest that it be any more "weedy" than the present corn cultivars that are the result of traditional breeding. The corn line MON 80100 has retained the agronomic characteristics of the parental corn. Monsanto company has provided data regarding seed germination rates, yield characteristics, disease and pest susceptibilities, compositional analyses, and numerous other tests reported in the Monsanto Company application that support APHIS' conclusion that corn line MON 80100 is just as safe to grow as any other insect resistant corn.

B. Potential impacts on the sexually-compatible relatives of corn arising from pollination by corn line MON 80100

Zea is a genus of the family Gramineae (the grass family) that consists of some 4 species: *Z. mays*, cultivated corn and teosinte; *Z. diploperennis*, diploperennial teosinte; *Z. luxurians*; and *Z. perennis*, a perennial teosinte. Of the four species of *Zea*, only *Z. mays* is common in the United States. It is known only from cultivation; it occasionally is spontaneous in abandoned fields or roadsides, but is incapable of sustained reproduction outside of cultivation (Gould 1968). The other species are occasional university or experiment station research subjects. *Z. perennis* is reported as established from James Island, South Carolina (Hitchcock and Chase 1951).

The closest relative to *Zea* is *Tripsacum*, a genus of seven species, three of which occur in the United States (Gould 1968). *Tripsacum* differs from corn in many respects, including chromosome number (n=9), in contrast to *Zea* (n=10). All species of *Tripsacum* can cross with *Zea*, but only with difficulty and resulting seeds are sterile (Galinat 1988).

Cultivated corn is presumed to have been transformed from teosinte, *Z. mays* subsp. *mexicana* more than 8000 years ago. During this transformation, cultivated corn gained several valuable agronomic traits, but lost the ability to survive in the wild. Teosinte, however, remains a successful wild grass in Mexico and Guatemala. Despite some confusion over proper taxonomic groupings of the non-cultivated members of *Zea*, wild members maintain a successful array of annual or perennial plants with visible chromosomal peculiarities and ploidy levels, and many adaptive macroscopic phenotypes. Cultivated corn and the wild members of diploid and tetraploid *Zea* can be crossed to produce fertile F_1 hybrids. Nonetheless, in the wild, introgressive hybridization does not occur because of differences in geographic separation, genetic structure, developmental morphology and timing of the reproductive structures, seed dissemination, and dormancy (Galinat 1988).

The second major transformation of cultivated corn occurred in the United States in the twentieth century, and particularly since the 1930's. This transformation occurred through the development of inbred lines for hybrid seed production, and by other methods. Almost all corn grown in the United States now comes from hybrid seed that is obtained every planting season from private enterprises; the older open-pollinated varieties are now virtually unknown in commerce (Hallauer et al. 1988). This transformation has resulted in more uniform commercial plants with superior agronomic characteristics, and has contributed to the six-fold increase in yields in the last sixty years.

Our analysis of the biology of cultivated lepidopteran insect resistant corn and its relatives leads us to predict that the environmental impacts of cultivation of corn line MON 80100 anywhere in the world would be no different from such impacts attributable to similar varieties produced with traditional breeding techniques. The species *Z. mays* is native to Mexico and central America. Non-cultivated varieties of *Zea* sp. have coexisted and co-evolved in the Americas over millennia. Even if corn line MON 80100 were to be cultivated in agricultural regions around centers of *Zea* diversity, there is no reason to expect impacts from corn line MON 80100 to be significantly different from those arising from the cultivation of any other variety of insect resistant corn.

International traffic in corn line MON 80100 would be fully subject to national and regional phytosanitary standards promulgated under the International Plant Protection convention (IPPC). The IPPC has set a standard for the reciprocal acceptance of phytosanitary certification among the nations that have signed or acceded to the Convention (98 countries as of December 1992). The treaty, administered by a Secretariat housed with the United Nations Food and Agriculture Organization in Rome, came into force on April 3, 1952. It establishes standards to facilitate the safe movement of plant materials across international boundaries. Plant biotechnology products are fully subject to national legislation and regulations, or regional standards and guidelines promulgated under the IPPC. The vast majority of IPPC signatories have promulgated, and are now administering, such legislation or guidelines. This includes Mexico, which has in place a regulatory process requiring a full evaluation of corn line MON 80100 before it can be introduced into their environment. The IPPC has also led to the creation of Regional Plant Protection Organizations (RPPOs) such as the North American Plant Protection Organization (NAPPO). Our trading partners will be kept informed of our regulatory decisions through NAPPO and other fora. Our decision in no way prejudices regulatory action in any other country.

It should be noted that all the considerable, existing national and international regulatory authorities and phytosanitary protocols that currently apply to introductions of new lepidopteran insect resistant corn varieties internationally apply equally to those covered by this analysis.

C. Potential impact on beneficial and other nontarget organisms

Consistent with its statutory authority and requirements under NEPA, APHIS evaluated the potential for corn line MON 80100 plants and plant products to have damaging or toxic effects directly or indirectly on nontarget organisms. This includes those that are recognized as beneficial to agriculture and to those that are recognized as threatened or endangered in the United States. APHIS also considered potential impacts on other "nontarget" pests, since such impacts could have an impact on the potential for changes in agricultural practices. There is no reason to believe that deleterious effects or significant impacts on nontarget organisms, including beneficial organisms, would result from the *EPSPS* and *gox* genes used as selectable markers during development of this line.

APHIS evaluated the results of several studies designed to compare the impact on nontarget organisms of corn line MON 80100 and *cryIA(b)* gene. In these studies trypsin-resistant full length CryIA(b) core protein purified from *E. coli* was used. About ten times of the concentration of the CryIA(b) core protein that killed target lepidoptera (2 ppm) was fed to the test organisms. The CryIA(b) protein was expressed in the corn leaf (1.3 ppm) and seed (0.57 ppm) as measured by the immunoassay.

1. Honey bee larvae and adults

Studies assessed the potential toxicity of the CryIA(b) trypsin-resistant core protein to larvae and adult honey bee (*Apis mellifera* L.), a beneficial insect pollinator. The protein concentration greater than 10 times the amount that killed target Lepidoptera had no visible effect on honey bees or their larvae.

2. Green lacewing (*Chrysopa carnea*)

Larvae of the beneficial predatory insects, green lacewing, were not adversely effected when fed for seven days moth eggs coated with about eight times concentration (16.7 ppm) of CryIA(b) core protein that kills ECB (Hoxter and Lynn, 1992a).

3. Parasitic hymenoptera

Brachymeria intermedia is a beneficial parasite of the housefly (*Musca domestica).* Parasitic Hymenoptera exposed to ten times the concentration of activated CryIA(b) protein that kills ECB (20 ppm in honey/water solution) for thirty days did not exhibit treatment related mortality or signs of toxicity (Hoxter and Lynn, 1992c).

4. Ladybird beetles

This study was undertaken to assess the potential toxicity of CryIA(b) trypsin-resistant core protein to ladybird beetles (*Hippodamia convergens*), a beneficial predaceous insect which feeds on aphids and other plant insects commonly found on stems and foliage of weeds and cultivated plants. Ladybird beetles exposed to activated CryIA(b) protein at a test concentration of 20 ppm in a honey/water solution for nine days did not exhibit treatment related mortality or signs of toxicity (Hoxter and Lynn, 1992b, MacIntosh *et al. 1990*).

5. Quail

A study was conducted to assess the wholesomeness of insect protected corn meal fed to quail since birds may feed on corn left in the field after harvest. No mortality occurred in birds fed up to 10% (nominal 100,000 ppm) raw corn seed meal in the diet. Based on the parameters measured, the wholesomeness of meal from insect protected corn seed was comparable to that of the parental line when fed in the diet to quail. Consumption of diet containing 10% of the diet on weight basis of raw corn meal is equivalent to the quail eating 138 seeds/kg body weight per day. The seeds contain 0.57 ppm of the CryIA(b) protein (Campbell and Beavers, 1994).

6. Impact on Endangered Species

No endangered or threatened lepidopteran insects, as listed in 50CFR 17.11 and 17.12, feed on corn plants.

Other invertebrates and all vertebrate organisms, including non-target birds, mammals and humans, are expected to be unaffected by the *Btk* insect control protein, because they would not be expected to contain the receptor protein found in the midgut of target insects.

7. Potential impact on threatened and endangered arthropods

The host ranges and habitats of the lepidopteran insect species currently listed or proposed as threatened or endangered in the U.S. were examined to determine if corn line MON 80100 might have an adverse impact on these species. None of these species inhabit corn fields or feed on corn. Most of the endangered species usually occur in specialized habitats. Often the habitat or unique plant that these butterflies or moths require for a successful life cycle is disappearing or threatened by human activities (BBEP-EAD National Endangered Species Database, 1994). For example, Smith's blue butterfly lives in coastal regions around Monterey County, California. Survival of this butterfly is dependent on its larval host food plants, and seacliff buckwheat. The primary factor limiting the Smith's butterfly is distribution of the two host plants.

APHIS concludes that corn line MON 80100 will not have a significant adverse impact on organisms beneficial to plants or agriculture, nontarget organisms, and will not harm threatened or endangered species.

D. Potential impacts on agricultural and cultivation practices

Although chemical insecticides (organophosphates and pyrethroids) and foliar applied *Btk* formulations can be effective against ECB, they must be applied before the insect bores into the stalk. Repeat applications are often necessary. If commercialized, corn line MON 80100 could offer an important alternative to chemical insecticides. Only about 5 percent of corn acreage in the U.S. is treated with foliar *Btk* products. By the same token, widespread and inappropriate use of either corn line MON 80100 or increased use of foliar microbial *Btk* products can and will most likely accelerate the appearance of ECB populations resistant to the *Btk* insect control protein. The rate with which resistance will develop using either strategy is difficult to predict. The rate depends on many assumptions regarding resistance management strategies, their acceptance and effective implementation by growers, the genetics of ECB resistance to this insecticide, and population and behavioral biology of ECB (Tabashnik 1994a,b; Gould et al. 1994).

The implementation of an active resistance management plan that is scientifically sound and acceptable to growers should delay the onset of resistance and provide alternative strategies and methods for managing or containing resistant populations if they occur. Monsanto Company has implemented strategies to: (1) develop genes for new insect control proteins, (2) monitor ECB susceptibilities to corn line MON 80100 plants, and (3) conduct and support research relevant to ECB resistance management. Monsanto Company has stated that it is in their best interest to delay resistance. The EPA has stated that they will work with Monsanto Company to develop product labels and informational brochures that are consistent with resistance management, and this should help define the appropriate use of corn line MON 80100.

If resistant populations persist, insecticides based on the CryIA(b) insect control protein would no longer be effective for controlling ECB on corn (ECB is not a serious pest of other crops). To control resistant ECB populations, producers could use currently registered insecticides, possible including new delta-endotoxins.

Based on this analysis, APHIS concludes that there is unlikely to be any significant adverse impact on agricultural practices associated with the appropriate use of corn line MON 80100. Similar to the consequence of deployment of other insecticides, resistance development in insect pest populations is probable. However, cultivation of ECB-resistant corn plants should pose no greater threat to the ability to control ECB in corn, than that posed by the insecticides already in use.

E. Corn line MON 80100 will not cause damage to processed agricultural commodities

The components and processing characteristics of corn line MON 80100 reveal no differences in any component that would likely have an indirect plant pest effect on any processed plant commodity.

VI. Conclusion

APHIS has evaluated information from the scientific literature as well as data submitted by Monsanto Company that characterized corn line MON 80100. After careful analysis, APHIS has identified no significant impact to the environment from issuance of a determination that corn line MON 80100 should no longer be a regulated article under APHIS regulations at 7 CFR Part 340. That finding is supported by the following conclusions:

1. Corn line MON 80100 exhibits no plant pathogenic properties. Although pathogenic organisms were used in their development, these corn plants are not infected by these organisms nor can these plants incite disease in other plants.

2. Corn line MON 80100 is no more likely to become a weed than insect-resistant corn developed by traditional breeding or other techniques. Corn is not a serious, principal or common weed pest in the U.S. There is no reason to believe that resistance to insects would enable corn to become a weed pest.

3. Multiple factors act to make gene introgression from corn line MON 80100 into wild or cultivated sexually-compatible plants extremely unlikely. Such rare events should not increase the weediness potential of any resulting progeny or adversely impact biodiversity.

4. Seeds of corn line MON 80100 are substantially equivalent in composition, quality and other characteristics to nontransgenic corn and should have no adverse impacts on raw or processed agricultural commodities.

5. Corn line MON 80100 poses no harm to threatened or endangered species, exhibits no significant potential either to harm organisms beneficial to agriculture or to impair the ability of farmers to control nontarget insect pests.

6. Cultivation of corn line MON 80100 should not reduce the ability to control insects in corn and other crops.

Therefore, after a review of the available evidence, APHIS concludes that there will be no significant

impact on the human environment if corn line MON 80100 were no longer considered a regulated article under regulations at 7 CFR Part 340. APHIS has also concluded that there is a reasonable certainty that new varieties developed from corn line MON 80100 will not exhibit new plant pest properties, i.e., properties substantially different from any observed in the field tested MON 80100 corn line, or those observed in corn in traditional breeding programs.

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Response to Monsanto Company Petition for Determination of Nonregulated Status for Insect-protected Corn line MON 80100

Prepared by
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I. SUMMARY

APHIS regulations promulgated pursuant to authority granted by the Federal Plant Pest Act (FPPA), and the Plant Quarantine Act (PQA), govern the introduction (importation, interstate movement, or release into the environment) of certain genetically engineered organisms and products. An organism is no longer subject to these regulatory requirements when it is demonstrated not to present a plant pest risk. The regulations provide 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.

Based on a review of scientific data and literature, the Animal and Plant Health Inspection Service (APHIS) has determined that lepidopteran insect-protected corn (hereafter referred to as corn line MON 80100) does not present a plant pest risk and is therefore no longer a regulated article. APHIS made this determination in response to a petition received on April 3, 1995 from Monsanto Company. The petition requested a determination from APHIS that corn line MON 80100 does not pose a plant pest risk and therefore, is not a regulated article. APHIS announced receipt of the petition in the *Federal Register* (60 *FR* 30061-30062) on June 7, 1995 and stated that the petition was available for public view. APHIS invited written comments on this proposed action, to be submitted on or before August 7, 1995. As a result of this determination, oversight under 7 CFR Part 340 will no longer be required by APHIS for field testing, importation, or interstate movement of corn line MON 80100 or its progeny.

Corn line MON 80100 is genetically engineered to carry a gene that codes for an insecticidal protein naturally produced by the soil bacterium *Bacillus thuringiensis* subsp. *kurstaki* (*Btk*), together with a selectable marker genes encoding phosphinothricin acetyltransferase from *Streptomyces hygroscopicus*. These genes also have accompanying DNA regulatory sequences that modulate their expression. The regulatory sequences were derived from corn and the plant pathogenic cauliflower mosaic virus (CaMV) and *Agrobacterium tumefaciens*.

This determination has been made based on an analysis that revealed that corn line MON 80100 plants: 1) exhibit no plant pathogenic properties, 2) are no more likely to become a weed than insect-resistant corn developed by traditional breeding, 3) are unlikely to increase the weediness potential of any other cultivated plant, 4) do not cause damage to processed agricultural commodities, 5) are unlikely to harm other organisms that are beneficial to agriculture or to adversely impact the ability to control nontarget insect pests, and 6) are unlikely to reduce the ability to control insects in corn and other crops. APHIS has also concluded that there is no reason to believe that new corn varieties derived from corn line MON 80100 progeny will exhibit new plant pest properties; i.e., properties substantially different from any observed for the corn line MON 80100 already field tested, or those observed for corn in traditional breeding programs.

The potential environmental impacts associated with this determination have been examined in accordance with regulations and guidelines implementing the National Environmental Policy Act (NEPA) of 1969 (42 U.S.C. 4331 *et seq.*; 40 CFR 1500-1509; 7 CFR Part 1b; 44 FR 50381-50384; and 44 FR 51272-51274). An environmental assessment (EA) was prepared and a preliminary Finding of No Significant Impact (FONSI) was reached by

APHIS for the determination that corn line MON 80100 is no longer a regulated article under its regulations at 7 CFR Part 340. This decision does not release corn line MON 80100 from regulations administered by the Environmental Protection Agency (EPA) under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136 *et seq.*) and the Federal Food, Drug, and Cosmetic Act (FFDCA) (21 U.S.C. 301 *et seq.*).

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

II. BACKGROUND

A. APHIS Regulatory Authority

APHIS regulations at 7 CFR 340, which were promulgated pursuant to authority granted by the Federal Plant Pest Act (FPPA), (7 U.S.C. 150aa-150jj) as amended, and the Plant Quarantine Act (PQA), (7 U.S.C. 151-164a, 166-167) as amended, regulate the introduction (importation, interstate movement, or release into the environment) of certain genetically engineered organisms and products. Under this regulation, a genetically engineered organism is deemed a regulated article either if the donor organism, recipient organism, vector or vector agent used to develop the organism belongs to one of the taxa listed in the regulation and is also a plant pest; or if APHIS has reason to believe that the genetically engineered organism presents a plant pest risk. The FPPA gives U.S. Department of Agriculture (USDA) the authority to regulate plant pests and other articles to prevent direct or indirect injury, disease, or damage to plants and plant products. In addition, the PQA provides an additional level of protection by enabling USDA to regulate the importation and movement of nursery stock and other plants that may harbor injurious pests or diseases.

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

An organism is not subject to the regulatory requirements of 7 CFR Part 340 when it is demonstrated not to present a plant pest risk. Section 340.6 of the regulations, entitled "Petition Process for Determination of Nonregulated Status," provides that a person may petition the agency to evaluate submitted data and determine that a particular regulated article does not present a plant pest risk and should no longer be regulated. If the agency

determines that the regulated article does not present a risk of introduction or dissemination of a plant pest, the petition will be granted, thereby allowing for unregulated introduction of the article in question. A petition may be granted in whole or in part.

The Corn line MON 80100 has been considered a "regulated article" for field testing under Part 340 of the regulations in part because certain noncoding regulatory sequences were derived from CaMV and *Agrobacterium tumefaciens*, known plant pests. APHIS believes it prudent to provide assurance before commercialization that organisms such as corn line MON 80100, which are derived at least in part from plant pests, do not pose any potential plant pest risk. Such assurance may aid the entry of new plant varieties into commerce or into breeding and development programs. The decision by APHIS that Corn line MON 80100 is not a regulated article is based in part on evidence provided by Monsanto Company concerning the biological properties of corn line MON 80100 and its similarity to other varieties of corn grown using standard agricultural practices for commercial sale or private use.

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

A determination that such insect-protected organisms do not present a plant pest risk can be made under this definition, especially when there is evidence that the plant under consideration: 1) exhibits no plant pathogenic properties; 2) is no more likely to become a weed than insect-protected corn developed by traditional breeding; 3) is unlikely to increase the weediness potential of any other cultivated plant; 4) does not cause damage to processed agricultural commodities; 5) is unlikely to harm other organisms that are beneficial to agriculture or to adversely impact the ability to control nontarget insect pests; and 6) is unlikely to reduce the ability to control insects in corn and other crops. Evidence has been presented by Monsanto Company that bears on these topics. In addition, it should be established that there is no reason to believe that any new corn varieties bred from corn line MON 80100 will exhibit plant pest properties substantially different from any observed for corn in traditional breeding programs, or as seen in the development of corn line MON 80100 already field tested. APHIS does anticipate that there will be new corn varieties bred from corn line MON 80100.

B. EPA and FDA regulatory authority

The corn line MON 80100 lines are currently subject to regulations administered by the EPA and/or the FDA (described in Section II.C. of the Environmental Assessment) that require registration of pesticides prior to their distribution and sale and establish tolerances for pesticide residues in raw agricultural products. APHIS' decision on the regulatory status of the corn line MON 80100 under APHIS' regulations at 7 CFR 340, in no way releases this corn and its progeny from EPA and FDA regulatory oversight.

III. RESPONSE TO COMMENTS

APHIS received nine comments on the Monsanto petition from farmers, industry, and a university research center. All the commenters expressed support for the subject petition for nonregulated status.

IV. ANALYSIS OF THE PROPERTIES OF CORN LINE MON 80100

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

Zea mays Linnaeus, known as maize throughout most of the world, and as corn in the United States, is a large, annual, monoecious grass, that is grown for animal feed, silage, vegetable oil, sugar syrups, and other uses. Corn is grown commercially in almost all States of the United States (Jewell, 1989). Corn has been cultivated since the earliest historic times from Peru to central North America. The region of origin is now presumed to be Mexico (Gould, 1968). Dispersal to the Old World is generally deemed to have occurred in the sixteenth and seventeenth centuries (Cobley and Steele, 1976); however, recent evidence indicates that dispersal to India may have occurred prior to the twelfth and thirteenth centuries by unknown means (Johannessen and Parker, 1989).

Zea is a genus of the family Gramineae (Poaceae), commonly known as the grass family. The genus consists of four species: *Z. mays*, cultivated corn and teosinte; *Z. diploperennis* Iltis et al., diploperennial teosinte; *Z. luxurians* (Durieu et Asch.) Bird; and *Z. perennis* (Hitchc.) Reeves et Mangelsd., perennial teosinte. Variations of the species have been assigned to the segregate genus *Euchlaena*, which is not currently recognized, or have been divided into numerous small species within the genus *Zea* (Terrell et al., 1986). Of the four species of *Zea*, only *Z. mays* is common in the United States. It is known only from cultivation; it occasionally is spontaneous in abandoned fields or roadsides, but is incapable of sustained reproduction outside of cultivation (Gould, 1968). The other species are occasional university or experiment station research subjects. *Z. perennis* is reported as established from James Island, South Carolina (Hitchcock and Chase, 1951).

The closest generic relative to *Zea* is *Tripsacum*, a genus of seven species, three of which occur in the United States (Gould, 1968). *Tripsacum* differs from corn in many respects,

including chromosome number (n=9), in contrast to *Zea* (n=10). All species of *Tripsacum* can cross with *Zea*, but only with difficulty and all lead to sterile progeny (Galinat, 1988).

Cultivated corn is presumed to have been transformed from teosinte, *Z. mays* subspecies *mexicana* (Schrader) Iltis, more than 8000 years ago. During this transformation, cultivated corn gained several valuable agronomic traits, but lost the ability to survive in the wild. Teosinte, however, remains a successful wild grass in Mexico and Guatemala. Despite some confusion over proper taxonomic groupings of the non-cultivated members of *Zea*, the wild members of the genus, whether annual or perennial exhibit an array of visible chromosomal peculiarities and ploidy levels, and many adaptive macroscopic phenotypes. Cultivated corn and wild diploid and tetraploid members of *Zea* can be crossed to produce fertile F_1 hybrids. Nonetheless, in the wild, introgressive hybridization does not occur because of differences in flowering time, geographic separation, block inheritance, developmental morphology, seed dissemination, and dormancy (Galinat, 1988).

The second major transformation of cultivated corn in the United States started in the 1930's and continues today through the development and use of inbred lines for hybrid seed production. Almost all corn grown in the United States now comes from hybrid seed that is obtained every planting season from private enterprises; the older open-pollinated varieties are now virtually unknown in commerce. This transformation has resulted in more uniform commercial plants with superior agronomic characteristics, and has contributed to the six-fold increase in yields over the last sixty years.

A variety of studies were conducted to characterize the unique traits of the modified corn line and to establish that insect protected corn line corn line MON 80100 is substantially equivalent to non-modified corn.

The CryIA(b) Protein

The CryIA(b) protein must be ingested by the insect to exert insecticidal activity (Huber and Lüthy, 1981). The protein in its crystalline form is insoluble in aqueous solution at neutral or acidic pH (Bulla *et al*, 1977); however, the alkaline pH of the larval insect gut dissolves the protein crystal. The dissolved protein is activated by proteases in the insect gut. The activated protein, approximately 600 amino acids long, diffuses through the peritrophic membrane of the insect to the midgut epithelium, binding to the specific high affinity receptors on the surface of the midgut epithelium of target insects (Wolfersberger *et al*, 1986; Hofmann *et al*, 1988a). The gut becomes paralyzed as a consequence of changes in electrolytes and pH gut causing the larval insect to quit feeding and die.

There are no receptors for the *Bt* protein delta-endotoxins on the surface of mammalian intestinal cells. Humans are therefore not susceptible to these proteins (Hofmann *et al*, 1988b; Noteborn, 1994; Sacchi *et al*, 1986) but digest them like other proteins. In addition to the lack of receptors for the *Btk* proteins, the absence of adverse effects in humans is further supported by numerous reviews on the safety of the *Bt* protein (Ignoffo, 1973; Shadduck, 1983; Siegel and Shadduck, 1989) and by rodent feeding (Naylor, 1992) and *in vitro* digestive fate studies of the *Btk* CryIA(b) protein (Ream, 1994).

Data were submitted to the EPA to support the registration and exemption from the requirement of a tolerance for the CryIA(b) protein as a plant pesticide. Studies included within that submission demonstrate the safety of this protein. In a mouse acute oral gavage study, no treatment related effects were observed in any of the groups of mice administered the CryIA(b) trypsin-resistant core protein by oral gavages at dosages up to 4000 mg/kg. The oral LD_{50} for the CryIA(b) protein in mice is greater than 4000 mg/kg and the no effect level is 4000 mg/kg (Naylor, 1992).

In an *in vitro* mammalian digestion study, the CryIA(b) protein degraded rapidly; more than 90% of the initially added CryIA(b) protein degraded after two minutes incubation in simulated gastric fluid as detected by western blot analysis and insect bioassay. In intestinal fluid, the trypsin-resistant core of the CryIA(b) protein did not degrade substantially after approximately 19.5 hours incubation as assessed by both western blot analysis and by insect bioassay (Ream, 1994). This result was expected as the trypsin-resistant core of this and other *Bt* insecticidal proteins have been shown to be relatively resistant to digestion by trypsin (Bielot *et al*, 1989). These results are fully consistent with the history of safe use of *Bt* preparations by humans.

The EPSPS Protein

EPSPS is an enzyme of the shikimate pathway for aromatic amino acid biosynthesis in plants (including corn) and microorganisms (Levin and Sprinson, 1964; Steinrüken and Amrhein, 1980), and is thus ordinarily present in food derived from plant sources. Genes for numerous EPSPS's have been cloned (Padgette *et al*, 1989, 1991), and active site domains are conserved among the known EPSPSs (Padgette *et al*, 1988, 1991). Bacterial EPSPSs have been well-characterized with respect to the 3-dimensional X-ray crystal structure (Stallings *et al*, 1991) and the chemical reaction mechanism (Anderson *et al*, 1990). EPSPSs from a number of bacteria exhibit tolerance to glyphosate (Schulz *et al*, 1985).

The herbicide glyphosate kills plants cells due to inhibition of the enzyme EPSPS (Steinrücken and Amrhein, 1980). The aromatic amino acid pathway is not present in mammalian metabolic pathways (Cole, 1985). This contributes to the selective action of glyphosate toward plants but not mammals. Glyphosate tolerance can be conferred to plant cells and microbes by either overproduction of EPSPS or the use of glyphosate-tolerant EPSPSs. The EPSPS from *Agrobacterium* sp. strain CP4 is highly tolerant to inhibition by glyphosate and has high catalytic efficiency, compared to most glyphosate-tolerant EPSPSs (Barry *et al*, 1992; Padgette *et. al.*, 1991). Upon glyphosate treatment, the corn cells in the transformation process expressing the CP4 EPSPS are unaffected since the continued action of the glyphosate-tolerant EPSPS enzyme meets the plant's need for aromatic compounds.

CP4 EPSPS is a 47.6 kilodalton (KD) protein consisting of a single polypeptide of 455 amino acids. The gene encoding CP4 EPSPS has been completely sequenced. The enzyme has been expressed in *E. coli* and highly purified. CP4 EPSPS interacts with the EPSPS substrates shikimate-3-phosphate and phosphoenolpyruvate similarly to the plant enzymes, based on steady-state kinetic analyses. In addition, recent results indicate that the 3-dimensional X-ray crystal structure of CP4 EPSPS exhibits the same overall folding pattern

as the *E. coli* EPSPS enzyme. The isolate CP4 was identified by the ATCC (American Type Culture Collection) as an *Agrobacterium* species, hence the designation *Agrobacterium* sp. strain CP4. Agrobacteria occur almost worldwide in soils and in the rhizosphere of plants. *Agrobacterium* strains have also been reported in a number of human clinical specimens, but it is believed that these clinical *Agrobacterium* isolates occur either as incidental inhabitants in the patient or as contaminants introduced during sample manipulation (Kersters and De Ley, 1984).

The chloroplast transit peptide (CTP) coding sequence from petunia EPSPS (Shah *et al*, 1986; Gasser *et al*, 1988) has been fused to the 5'-end of the CP4 EPSPS gene to deliver the CP4 EPSPS to the chloroplasts, the site of EPSPS activity and glyphosate action. Plant expression of the gene fusion produces a pre-protein which is rapidly imported into chloroplasts where the CTP is cleaved and degraded, releasing the mature CP4 EPSPS protein (della-Cioppa *et al*, 1986).

Expression Levels of the CryIA(b), CP4 EPSPS, GOX, and NPTII Proteins

Insect protected corn line MON 80100 has been modified to express a protein from *Bacillus thuringiensis* subsp. *kurstaki* strain CryIA(b) (Höfte and Whitely, 1989). This protein has insecticidal activity against the European corn borer (ECB), an economically damaging corn insect pest. In addition to the *cryIA(b)* gene, genes encoding CP4 EPSPS (Padgette *et al*, 1993), GOX (Padgette *et al*, 1994), and NPTII are also present. The CP4 EPSPS and GOX genes are present to enable selection of cells in tissue culture that contain the *cryIA(b)* gene. The corn transformation vectors used to produce corn line corn line MON 80100 included the gene cassette containing a bacterial specific promoter and coding region for NPTII. NPTII allows selection of bacteria containing the vector in media containing kanamycin. The *npt*II gene is under the control of a bacterial-specific promoter and, therefore, is not expected to produce the NPTII protein in plant cells.

A. The introduced genes, their products, and the added regulatory sequences do not present a plant pest risk in corn line MON 80100.

Two plasmids, PV-ZMBK07 and PV-ZMGT10, were transformed in corn genotype Hi-II by particle acceleration transformation system to generate corn line MON 80100.

The plasmid vector PV-ZMBK07 contains the *cryIA(b)* gene under the control of the enhanced 35S promoter (E35S) (Kay *et al*, 1985 and Odell *et al*, 1985), which is approximately 0.6 Kb in size. Located between the enhanced 35S promoter and the *cryIA(b)* gene is the 0.8 Kb intron from the *hsp70* gene (heat-shock protein), present to increase the levels of gene transcription (Rochester *et al*, 1986). The *cryIA(b)* gene is joined to the 0.24 Kb nopaline synthase 3' nontranslated sequence, NOS 3', (Rogers *et al*, 1985) which provides the mRNA polyadenylation signals. The *cryIA(b)* gene is 3468 nucleotides in length and encodes a full-length *Btk* HD-1 [CryIA(b)] protein of 1156 amino acids, which when subjected to trypsin yields an active trypsin-resistant protein product of approximately 600 amino acids *in planta* and *in vitro* (Lee *et al*, 1995). The *cryIA(b)* gene sequence was modified to increase the levels of expression in corn using strategies similar to those as

previously described (Perlak *et al*, 1991). The gene encodes the nature identical CryIA(b) *Btk* HD-1 protein product (Fischhoff *et al*, 1987). The *alpha* region of the *lacZ* gene for beta-galactosidase, present under a bacterial controlled promoter, is present in PV-ZMBK07. This region contained a polylinker (region with multiple cloning sites) which allowed for the cloning of the desired genes within the plasmid vector (Vieira and Messing, 1987). Most of the approximately 200 bp region of the alpha region of the gene for beta-galactosidase was not incorporated into corn line corn line MON 80100. The *lacZ-alpha* region is followed by the 0.7 Kb origin of replication for the pUC plasmids (*ori-pUC*) and which allows for the replication of plasmids in *E. coli* (Vieira and Messing, 1987). Following the *ori-pUC* region is the gene for the enzyme neomycin phosphotransferase type II (NPTII). This enzyme confers resistance to aminoglycoside antibiotics (*i.e.*, kanamycin and neomycin) and was used for selection of bacteria during the construction of this plasmid. The coding sequence for the *nptII* gene was derived from the prokaryotic transposon Tn5 (Beck *et al*, 1982) and is present under its own bacterial promoter. The NPTII protein was not detected in corn line corn line MON 80100 (Ream *et al*, 1995).

The PV-ZMGT10 plasmid contains the *gox* and CP4 EPSPS genes joined to chloroplast transit peptides CTP1 and CTP2, respectively. Both coding regions are under the control of the enhanced 35S promoter, *hsp70* intron and NOS 3' terminator sequences. The PV-ZMGT10 vector contains the same *lacZ-alpha*, *ori-pUC* and *nptII* regions as described above for PV-ZMBK07. The CP4 EPSPS and *gox* genes enable the selection of cells in tissue culture that contain the *cryIA(b)* gene. A CP4 EPSPS has been isolated from *Agrobacterium* sp. strain CP4 which has been shown to be highly resistant to glyphosate (Harrison *et al*, 1993). The CP4 EPSPS protein represents one of many different EPSPSs found in nature (Schulz *et al*, 1985) and is highly tolerant to the inhibition by glyphosate and has high catalytic efficiency, compared to most EPSPSs (Barry *et al*, 1992; Padgette *et al*, 1991). Upon glyphosate treatment, plants or plant cells expressing the CP4 EPSPS protein are unaffected since the continued action of the tolerant EPSPS enzyme meets the plant's need for aromatic compounds. The CP4 EPSPS gene in PV-ZMGT10 contains a chloroplast transit peptide, CTP2, isolated from *Arabidopsis thaliana* EPSPS (Klee and Rogers, 1987) which directs the CP4 EPSPS protein to the chloroplast, the location of EPSPS in plants and the site of aromatic amino acid synthesis (Kishore and Shah, 1988). The CP4 EPSPS gene with its CTP2 is approximately 1.7 Kb in size. The CP4 EPSPS gene cassette (promoter through 3' termination sequence) is joined to the *gox* cassette.

The *gox* gene that encodes the glyphosate metabolizing enzyme glyphosate oxidoreductase (GOX) was cloned from *Achromobacter* sp. strain LBAA (Hallas *et al*, 1988; Barry *et al*, 1992; Barry *et al*, 1994). The GOX protein is targeted to the plastids with a chloroplast transit peptide sequence, CTP1. The CTP1 was derived from the small subunit gene of ribulose-1,5-bisphosphate carboxylase (SSU1A) gene from *Arabidopsis thaliana* (Timko *et al*, 1988). The enzyme GOX degrades glyphosate by converting glyphosate to aminomethylphosphonic acid (AMPA) and glyoxylate. The GOX protein was not detected by ELISA or western blot analysis in the seeds or leaves of corn line MON 80100.

The plant produced EPSPSs are present in the chloroplast. Therefore, the chloroplast transit peptide coding sequence, CTP2, from *Arabidopsis thaliana* EPSPS (Klee *et al*, 1987) was

fused to the N-terminus of the CP4 EPSPS protein to deliver the CP4 EPSPS to the chloroplasts, the site of EPSPS activity and glyphosate action (Kishore and Shah, 1988). CTPs are typically cleaved from the "mature" protein following delivery to the plastid (della Cioppa *et al*, 1986). The bacterial selectable marker gene, *nptII* , isolated from the prokaryotic transposon, Tn5 (Beck *et al*, 1982), encodes for the enzyme neomycin phosphotransferase II which confers resistance to the aminoglycoside antibiotics (i.e., kanamycin or neomycin) used for selection of plasmids in *E. coli*. The promoter for this gene is only active in bacterial hosts. As expected, no NPTII protein was detected in the insect protected corn line corn line MON 80100 using western blot analysis.

B. Expression of insect control protein in the corn line MON 80100 will not likely provide a competitive advantage sufficient to cause these plants to become any more "weedy" than other corn.

APHIS evaluated whether the corn line MON 80100 is any more likely to become a successful weed than nontransgenic control corn. Most definitions of weediness stress the undesirable nature of weeds from the point of view of humans; individual definitions differ in approach and emphasis (Baker, 1965; Muenscher, 1980). Baker defines a plant as a weed if, in any specified geographical area, its populations grow entirely or predominantly in situations markedly disturbed by man (without, of course, being deliberately cultivated) (Baker, 1965). He also described the ideal characteristics of weeds as including the following: discontinuous germination and long-lived seeds; rapid seedling growth; rapid growth to reproductive stage; long continuous seed production; self-compatibility, but not obligatory self-pollination or apomyxis; if outcrossing, use of wind or an unspecialized pollinator; high seed output under favorable conditions; germination and seed production under a wide range of environmental conditions; high tolerance or plasticity of climatic and edaphic variation; special adaptations for dispersal; good competitiveness achieved through, for example, allelochemicals or choking growth; and, if perennial, then exhibiting vigorous vegetative reproduction, brittleness either at the lower nodes or of rhizomes or rootstocks, and having the ability to regenerate from severed rootstocks. Although Baker's characteristics have been criticized by some ecologists as nonpredictive, no more broadly accepted suite of characteristics has been defined by ecologists (Williamson, 1994). In our view, there is no formulation that is clearly superior at this time. Keeler (1989) and Tiedje et al. (1989) have adapted and analyzed Baker's list to develop admittedly imperfect guides to the weediness potential of transgenic plants. Both authors emphasize the importance of looking at the parent plant and the nature of the specific genetic changes. Cultivated corn, particularly the line CG00526 variety, lacks most of Baker's "weedy" characteristics (Keeler, 1989). Corn is not listed as a common, serious or principal weed or a weed of current or potential importance in the United States and/or Canada in most weed compendiums (Holm et al., 1991; Muenscher, 1955; USDA, 1971; Weed Science Society of America, 1992).

Expression of the insect control protein in the corn line MON 80100 will not likely provide a competitive advantage sufficient to cause these to be any more "weedy" than other corn cultivars. None of the characteristics of weeds described by Baker involved resistance or susceptibility to insects. More importantly, in addition to the analysis above, APHIS evaluated field data submitted by Monsanto Company which demonstrate that corn line MON 80100 is no more weedy than any other corn. Control and corn line MON 80100 plants

were routinely monitored during field trials for differences in morphological characteristics, disease and insect susceptibility.

Based on evaluation of the available literature and data submitted by Monsanto Company, APHIS concludes that the corn line MON 80100 is no more likely to present a plant pest risk as a weed than are nontransgenic controls.

C. Gene introgression from corn line MON 80100 into wild or cultivated sexually-compatible plants is unlikely, and such rare events should not increase the weediness potential of resulting progeny or adversely impact biodiversity.

APHIS evaluated the potential for gene flow from corn line MON 80100 to other cultivated and wild relatives. Then two potential impacts that might result from this sexual transfer of genes were evaluated: first, that the traits from corn line MON 80100 might cause free-living relatives to become "weedier", and second, that the transfer of genes might cause population changes that would lead to reduced genetic diversity. The glyphosate resistance trait used as a selectable marker in the corn line MON 80100 was considered not to pose a hazard in this analysis. Glyphosate is already registered for use in corn in special application manner.

Gene introgression into other corn cultivars via cross pollination is possible. If pollen of the corn line MON 80100 was transferred by wind or any other means to receptive corn stigma within the 30 minute period of pollen viability, cross-pollination could occur. The potential for such transfer decreases as distance increases from the transgenic plants. From a practical standpoint, it becomes increasingly unlikely at distances much beyond the foundation seed isolation distance of 660 feet. Farmers purchase hybrid corn seed for planting from a commercial source. If pollen of corn line MON 80100 fertilizes corn in the fields of a farmer, any resulting corn seed will likely be used for some thing other than seed (eg oil or meal). Fertilization of nontransgenic corn by pollen from corn line MON 80100 in fields grown for sale as food or feed will not result in dissemination of the trait in to seed populations used for replanting. Moreover, seeds resulting from such fertilization will not themselves express the introduced genetic material.

As stated in Section IV., Monsanto Company reported no obvious differences in the flowering of corn line MON 80100 compared to the nontransgenic parent. There is no reason to believe that the genetic construct introduced during the transformation event would have any effect on the reproductive biology of the corn line MON 80100, unless the insertion event interrupted a genetic locus critical for the normal reproductive function.

Breeder seed is usually derived from self-pollinated seed at around the eighth to tenth generations of inbreeding (Wych, 1988). A high degree of self-pollination is ensured by planting well isolated blocks that virtually guarantee random mating. Minimum isolation distances for foundation seed are one-eighth mile (660 feet) from the nearest contaminating source. Other safeguards, such as physical barriers or border trap rows, can further reduce the possibility of contamination. Fields are preferred that have not been recently planted in corn. This is to minimize the appearance of volunteer corn from a previous season. Corn appears as a volunteer in some fields and roadsides, but it does not become established outside of cultivation (Gould et al, 1994).

D. Use of corn line MON 80100 should have no more adverse impacts on raw or processed agricultural commodities than the parent corn.

During extensive field testing, the corn line MON 80100 exhibited the typical agronomic characteristics of the recipient, with the exception of the desired corn line MON 80100 phenotype conferred by the *Btk* insect control protein. In APHIS' opinion, the components, quality and processing characteristics of corn line MON 80100 reveal no differences that could have an indirect plant pest effect on any raw or processed plant commodity. None of the sequences introduced is associated with any disease specific property of the donor organism from which it was derived, and corn line MON 80100 exhibit no plant pest characteristics.

E. Corn line MON 80100 exhibits no significant potential to harm organisms beneficial to agriculture, to harm threatened or endangered organisms or to have an adverse impact on the ability to control nontarget insect pests.

Organisms beneficial to the agricultural ecosystem: Consistent with its statutory authority and requirements under NEPA, APHIS evaluated the potential for corn line MON 80100 plants and plant products and the *Btk* insect control protein to have damaging or toxic effects directly or indirectly on nontarget organisms, particularly those that are recognized as beneficial to agriculture. APHIS also considered potential impacts on other nontarget pests, since such impacts could have an impact on the potential for changes in agricultural practices. There is no reason to believe that the *EPSPS* and GOX protein conferring glyphosate resistance in the corn line MON 80100 plants as a selectable marker for transformation would have deleterious effects or significant impacts on nontarget organisms, including beneficial organisms. Glyphosate herbicide, the target of the *EPSPS* gene product is not registered for use in corn. There have been no reports of toxic effects on such organisms in the many field trials conducted with many different plants expressing these selectable markers.

Data and information supplied by the applicant demonstrate that corn line MON 80100 is substantially equivalent to non-modified corn, except for the inserted genetic sequences, the expressed proteins [CryIA(b) protein, GOX and CP4 EPSPS enzymes], and the resulting ability of the plant to resist damage from certain Lepidopteran insects including European corn borer. Corn line MON 80100 is not likely to pose a greater plant pest risk than non-modified corn. This conclusion is based on evaluation of phenotypic characteristics, safety of the inserted proteins, and the lack of any deleterious environmental effects.

1. Non-target Insects

There is extensive information on the lack of non-target effects from microbial preparations of *Bacillus thuringiensis* subsp. *kurstaki* (*Btk*) containing the *Btk* proteins, including the CryIA(b) protein. The literature has established that the *Btk* proteins: are highly specific for lepidopteran insects (MacIntosh *et al*, 1990; Klausner, 1984; Aronson *et al*, 1986; Dulmage, 1981; Whitely and Schnepf, 1986). They bind specifically to receptors on the mid-gut of lepidopteran insects (Wolfersberger *et al*, 1986; Hofmann *et al*, 1988a; Hofmann *et al*, 1988b; Van Rie *et al*, 1989; Van Rie *et al*, 1990); and have no deleterious effect on

beneficial/non-target insects, including predators and parasitoids of lepidopteran insect pests or honeybees (*Apis mellifera*) (Flexner *et al*, 1986; Krieg and Langenbruch, 1981; Cantwell *et al*, 1972; EPA, 1988; Vinson, 1989; Melin and Cozzi, 1989).

The chapters by Vinson (1989) and Melin and Cozzi (1989) provide comprehensive reviews of the extensive literature that has established the safety of the *Btk* microbes and encoded proteins to an array of beneficial insects. In addition, separate studies were undertaken to assess the potential toxicity of the CryIA(b) protein to other non-target insects.

a. Honey bee larvae and adults

These studies assessed the potential toxicity of the CryIA(b) trypsin-resistant core protein to larvae and adult honey bee (*Apis mellifera* L.), a beneficial insect pollinator. The maximum nominal CryIA(b) protein concentration tested was greater than 10 times the estimated LC_{50} sensitivity of several target pest Lepidoptera to the CryIA(b) protein. The LC_{50} for the CryIA(b) protein in larval and adult honey bee is greater than 20 ppm. The no observed effect level was 20 ppm (Maggi and Sims, 1994a, 1994b).

b. Green lacewing

This study assessed the potential toxicity of the CryIA(b) trypsin-resistant core protein to green lacewing larvae (*Chrysopa carnea*), a beneficial predaceous insect commonly found in corn and other cultivated plants. There was no evidence that green lacewing larvae were adversely effected when fed moth eggs coated with a nominal concentration of 16.7 ppm CryIA(b) protein for seven days. Under the conditions of the test, the LC50 was greater than 16.7 ppm CryIA(b) protein (Hoxter and Lynn, 1992a).

c. Parasitic hymenoptera

This study assessed the potential toxicity of the CryIA(b) trypsin-resistant core protein to parasitic Hymenoptera (*Brachymeria intermedia*), a beneficial parasite of the housefly (*Musca domestica)*. Parasitic Hymenoptera exposed to activated CryIA(b) protein at a concentration of 20 ppm in honey/water solution for thirty days did not exhibit treatment related mortality or signs of toxicity. The LC_{50} for CryIA(b) protein in parasitic Hymenoptera is greater than 20 ppm. The no-observed effect level was 20 ppm (Hoxter and Lynn, 1992c).

d. Ladybird beetles

This study evaluated the potential toxicity of CryIA(b) trypsin-resistant core protein to ladybird beetles (*Hippodamia convergens*), a beneficial predaceous insect which feeds on aphids and other plant insects commonly found on stems and foliage of weeds and cultivated plants. Ladybird beetles exposed to activated CryIA(b) protein at a test concentration of 20 ppm in a honey/water solution for nine days did not exhibit treatment related mortality or signs of toxicity. The LC_{50} for CryIA(b) protein in ladybird beetles is greater than 20 ppm. The no-observed effect level was 20 ppm (Hoxter and Lynn, 1992b).

Insect protected corn line MON 80100 also encodes the enzyme CP4 EPSPS as discussed above. EPSPS is an enzyme of the shikimate pathway for aromatic amino acid biosynthesis in plants (including corn) and microorganisms (Levin and Sprinson, 1964), and is thus ordinarily present in food derived from plant sources. Genes for numerous EPSPS's have been cloned (Padgette *et al*, 1989, 1991), and active site domains are conserved among the known EPSPSs (Padgette *et al*, 1988, 1991). Bacterial EPSPSs have been well-characterized with respect to the 3-dimensional X-ray crystal structure (Stallings *et al*, 1991) and the detailed kinetic and chemical reaction mechanism (Anderson *et al*, 1990). EPSPSs from a number of bacteria exhibit tolerance to glyphosate (Schulz *et al*, 1985). CP4 EPSPS thus represents one of many different EPSPSs found in nature. EPSPS is considered to be ubiquitous in nature since it is present in all plants and microorganisms. Therefore, all organisms which presently feed on plants and/or microbes, historically have been exposed to EPSPS.

2. Non-Target Wildlife and Fish

A study was conducted to assess the wholesomeness of insect protected corn meal fed to quail since birds may feed on corn left in the field after harvest. No mortality occurred in birds fed up to 10% w/w (nominal 100,000 ppm) raw corn seed meal in the diet. This feeding level approximates consumption of 138 corn seeds/kg body weight/ bird/day. The no-observed effect level was considered to be greater than 10% w/w. Based on the parameters measured, the wholesomeness of meal from insect protected corn seed was comparable to that of the parental line when fed in the diet to quail (Campbell and Beavers, 1994).

It is unlikely that fish in their natural environment would be exposed to corn seed. Based on the historical data demonstrating the safety of *Bt* proteins to fish and the unlikely event of exposure, no adverse effects are expected to fish from the use of the CryIA(b) protein as expressed in corn.

3. Impact on Endangered Species

No endangered or threatened lepidopteran insects, as listed in 50CFR 17.11 and 17.12, feed on corn plants.

Environmental Fate of the CryIA(b) Protein

Previous work has demonstrated the rapid loss of insecticidal activity of *Bacillus thuringiensis* protein crystals (active ingredient) derived from microbial preparations of lepidopteran-active species when incubated in the soil (Palm *et al*, 1994; Pruett *et al*, 1980; West, 1984). The CryIA(b) protein in insect protected corn line MON 80100 is present at low levels in the plant tissue remaining in the field after the harvest of the seed or silage. This corn plant material may be tilled into the soil or remain on the soil surface as is typically observed in zero tillage systems. The environmental fate of the CryIA(b) protein was determined by measuring the rate at which the bioactivity of the CryIA(b) protein

dissipated when added to soil as the purified protein and as a component of insect protected corn tissue.

Studies examined the dissipation rate of the CryIA(b) protein from three systems: 1) insect protected corn tissue without contact with soil, 2) insect protected corn tissue mixed into soil, and 3) purified CryIA(b) protein mixed into soil. The levels incorporated into the soil were greater than three fold higher than the maximum concentration expected under field conditions. CryIA(b) protein, added to soil as a component of tissue from insect protected corn had an estimated DT_{50} of 1.6 days. Bioactivity of insect protected corn tissue, incubated without soil contact, had an estimated DT_{50} of 25.6 days. Purified CryIA(b) protein, mixed into the soil, had as estimated DT_{50} of 8.3 days. This rate of dissipation of insecticidal activity is comparable to that observed with microbial *Bt* products.

Therefore, results of this study suggest that the CryIA(b) protein, as a component of post-harvest insect protected corn plants, will dissipate readily on the surface of (e.g. no-till), or when cultivated into the soil. The measured half-life of the purified *Btk* protein in soil is comparable to that measured for the microbial *Btk* preparations (Palm *et al*, 1994; Pruett *et al*, 1980; West, 1984).

Endangered lepidopterans may conceivably be sensitive to CryIA(b) protein, given that the protein is selectively toxic to certain lepidopteran species. Endangered lepidopterans include several species of moths and butterflies: the El Segundo blue butterfly, the primrose sphinx moth, Lange's metalmark butterfly, the Lotis blue butterfly, the Oregon silverspot butterfly, the San Bruno elfin butterfly, Schaus' swallowtail butterfly, and Smith's blue butterfly. These species are not found in areas where corn is commonly grown. Corn is not among the host plants for these lepidopterans. Unlike the exposure scenarios typical of conventional or microbial insecticides, an organism must actually consume maize tissue to receive any exposure to CryIA(b). Therefore, we conclude that threatened and endangered organisms will not be affected as a result of corn line MON 80100.

Host-range comparisons have not indicated any change in range of species susceptible to maize CryIA(b) compared to native CryIA(b). In its effects on insects, corn line MON 80100 is similar to the microbial insecticidal preparations that are already commercially sold. Both field testing and laboratory testing of maize CryIA(b) have indicated that nontarget beneficial insects are not likely to be affected by maize CryIA(b), so it is not likely that endangered dipterans, hymenopterans, or coleopterans would be affected.

Ability to control nontarget insect pests: In all of the studies outlined above, there is no evidence that exposure to the CryIA(b) protein expressed in either maize pollen or extracted from *Bt* maize leaves resulted in any toxic effect on the organism tested. Tested organisms include representative avian, aquatic invertebrates, and soil invertebrate species, and several nontarget insect species. Testing with insects known to be either susceptible or not susceptible to native CryIA(b) gave no indications of a changed host specificity for the maize expressed CryIA(b). A small field monitoring study on beneficial insects did not detect any effects on beneficial insect or insect prey species exposed to *Bt* maize. These results suggest that only lepidopterans susceptible to native CryIA(b) are likely to be affected by *Bt* maize *CryIA(b)*.

APHIS concludes that corn line MON 80100 exhibit no significant potential to adversely impact organisms beneficial to plants or agriculture or to adversely impact the ability to control nontarget insect pests of agriculture.

F. Cultivation of corn line MON 80100 should not reduce the ability to control insects in corn and other crops.

APHIS considered potential impacts associated with the cultivation of corn line MON 80100 on the current agricultural practices used to control insects. Monsanto Company also provided APHIS a copy of their strategy for maximizing the utility of these plants and delaying the development of insect-protected to the *Btk* insect control protein. The development of effective resistance management strategies is an ongoing process already submitted by the petitioner for EPA consideration, and APHIS will continue to offer comments and recommendations for technical improvements to the EPA and Monsanto Company to assist in this process. The EPA has stated that they are committed to working with Monsanto Company to develop product labels and informational brochures that include instructions on the proper use of the corn line MON 80100 that are consistent with resistance management.

Both chemical and microbial insecticides are currently used for control of ECB in corn. Among chemical insecticides organophosphates (Counter®, Dyfonate®, Lorsban®, Thimet®, Parathion®, Penncap®), pyrethroids (Ambush®, Pounce®, Capture®), carbamate (Furadan®) and others (Asana XL®) are used. Though organophosphates and pyrethroids can be effective against ECB, careful insect surveillance is required. Applications must be carefully timed to reach insect populations before the insects bore into the stalk, and repeated applications are often necessary.

B. thuringiensis var. *kurstaki* preparations are registered for use on corn, vegetables, cotton, deciduous nuts and fruits. As crystalline powder formulations, *Btk* has been used commercially as an insecticide under the trade name Dipel. Only 5% of the commercially grown corn is treated with *Btk* preparations to control ECB. ECB reduces corn yields by causing physical damage to the plant and ear that result in weakness of the stalk dropped ears, and damaged grain. Yield reductions due to ECB infestation are estimated to exceed $50 million annually in the State of Illinois alone. *Btk* is very effective in the laboratory against ECB. However, commercial *Btk* formulations are generally ineffective in controlling ECB on corn because topical applications of the powder do not reach the inside the corn stalks where the insects feed. Ciba has engineered the corn line MON 80100 to produce the *Btk* insecticide specifically in corn tissue where ECB feeds. The use of this corn should provide farmers a means of controlling a serious insect pest that is not easily controlled by current chemical pesticides. Other advantages include: (1) reducing the risks associated with environmental spills or misapplication of chemical insecticides; (2) eliminating unwanted effects on beneficial insect populations (which can be susceptible to conventional chemical applications); these beneficial insects can, in turn, further reduce the reliance upon chemical means of pest control; and (3) reducing the consumption of fossil fuels required to deliver chemical inputs by machinery.

Corn line MON 80100 could provide a more effective alternative for season-long control of ECB compared to the use of foliar microbial *Btk* products that currently only receive limited use. Monsanto Company field data indicate that corn line MON 80100 is more effective than some *Btk* formulations at reducing insect survival and egg-laying. The corn line MON 80100 alternative is particularly important where chemical insecticides are no longer effective due to insect resistance. Over the course of use of corn line MON 80100, resistant insects will probably evolve but to recommend a resistance management strategy is outside the scope of this analysis.

Monsanto Company transgenic corn line MON 80100 offers an alternative control method. Monsanto Company strategies for maximizing the utility of these plants while delaying the development of insect resistance to these plants include the following:

1. Promoting the incorporation of corn line MON 80100 into integrated pest management programs (IPM) that emphasize the use of cultural control practices, such as those described above, and judicious and selective use of additional insecticides only when pest populations reach the threshold for economic damage. They do not encourage the use of corn line MON 80100 as a stand-alone control measure.

2. Monitoring insect populations for *Btk* protein susceptibility so that development of resistance can be detected and management strategies altered accordingly.

3. High dose expression of *Btk* protein to control insect that are heterozygous for resistance alleles.

4. Deployment of other corn lines or other hosts as refugia for insects that are sensitive to the *Btk* insect control protein, in order to maintain susceptible alleles in the population.

5. Development of new insect control proteins with a distinct mode of action to be employed with the *Btk* protein.

6. Implementation of a grower education program to achieve items 1, 2, and 4 above.

APHIS evaluated the potential impact to agricultural practices associated with the use of corn line MON 80100 according to Monsanto Company strategy. As a result of Monsanto Company program to instruct growers on the use of cultural control practices and IPM, growers may be more likely to adopt these methods. However, growers will also need to be informed about the implementation of preferred refugia strategies and how these can be integrated with other cultural practices. Growers will be less likely to use chemical insecticides targeted at insect control, and this should reduce the risks associated with some of these insecticides. The use of corn line MON 80100 should increase safety to field workers and consumers, reduce toxicity to nontarget species, and lower rates of ground water contamination by insecticides. Corn line MON 80100 plants are not likely to eliminate completely the use of chemical insecticides, particularly when they may be needed to control

other serious pests. But perhaps they may encourage more selective use of insecticides against these pests.

Monsanto Company support of research in resistance development to corn line MON 80100 and their grower education plan, coupled with ECB population monitoring programs, could enable them to implement strategies to delay resistance and detect and possibly contain it when it occurs. It may be possible to control resistant ECB populations by the use of agronomic practices such as crop rotation and alternate insecticides.

APHIS concludes that development of resistance to insecticides is a potential associated with their use; but in this respect, cultivation of corn line MON 80100 should pose no greater effects on the control of insects in corn and other crops, than the widely practiced method of applying insecticides.

V. CONCLUSION

APHIS has determined that corn line MON 80100 and its progeny 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 corn line MON 80100 or their progeny. (Importation of corn line MON 80100 seeds capable of propagation is still, however, subject to the restrictions found in the Foreign Quarantine Notice regulations at 7 CFR Part 319.) This determination has been made based on data collected from these trials, laboratory analyses and literature references presented herein which demonstrate the following:

1. Corn line MON 80100 exhibits no plant pathogenic properties. Although pathogenic organisms were used in their development, these corn plants are not infected by these organisms nor can these plants incite disease in other plants.

2. Corn line MON 80100 is no more likely to become a weed than insect-protected corn which could potentially be developed by traditional breeding techniques. Corn is not a serious, principal or common weed pest in the U.S., and there is no reason to believe that resistance to insects would enable corn to become weed pests.

3. Multiple barriers insure that gene introgression from Corn line MON 80100 into wild or cultivated sexually-compatible plants is extremely unlikely, and such rare events should not increase the weediness potential of any resulting progeny or adversely impact biodiversity.

4. Seeds of corn line MON 80100 are substantially equivalent in composition, quality and other characteristics to nontransgenic corn and should have no adverse impacts on raw or processed agricultural commodities.

5. Corn line MON 80100 exhibits no significant potential either to harm organisms beneficial to the agriculture or to impair the ability of farmers to control nontarget insect pests.

6. Cultivation of corn line MON 80100 should not reduce the ability to control insects in corn and other crops.

APHIS has also concluded that there will be new varieties bred from corn line MON 80100; however, if such varieties were developed they are unlikely to exhibit new plant pest properties, i.e., properties substantially different from any observed for corn line MON 80100 already field tested, or those observed for corn developed from traditional breeding.

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DATE

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