I. Summary

The Animal and Plant Health Inspection Service (APHIS), United States Department of Agriculture (USDA), has prepared an Environmental Assessment (EA) in response to a petition (APHIS Number 04-362-01p) from Syngenta Seeds, Inc. (Syngenta) regarding the regulatory status of genetically engineered (transformed) corn rootworm resistant corn derived from transformation event MIR604. This corn is currently a regulated article under USDA regulations at 7 CFR Part 340, and as such, interstate movements, importations, and field tests of MIR604 corn have been conducted under permits issued or notifications acknowledged by APHIS. Syngenta petitioned APHIS requesting a determination that MIR604 corn does not present a plant pest risk, and therefore MIR604 corn and its progeny derived from crosses with other non-regulated corn should no longer be regulated articles under these APHIS regulations.

II. Introduction

Syngenta has submitted a "Petition for Determination of Non-regulated Status" to the USDA/APHIS (APHIS number 04-362-01p) for genetically engineered corn plants that are resistant to the feeding damage caused by: the northern corn rootworm (NCRW, *Diabrotica longicornis barberi* Smith and Lawrence); the western corn rootworm (WCRW, *D. virgifera virgifera* Le Conte); and the Mexican corn rootworm (MCRW, *D. virgifera zeae* Krysan and Smith). The corn rootworm (CRW) larvae damage corn by feeding on the roots of corn plants, thereby inhibiting the ability of the plant to absorb water and nutrients from the soil (Reidell, 1990). This leads to harvesting difficulties due to lodging of the weakened plants (Spike and Tollefson, 1991). Annual losses to growers because of CRW have been estimated to approach a billion dollars when taking into account both the costs of chemical controls and crop losses from CRW (USDA-ARS, 2003).

Bacillus thuringiensis bacteria produce a group of related toxins (delta-endotoxins) that when ingested by susceptible insects (such as coleopterans and lepidopterans) result in insect death. Preparations of Bt containing delta-endotoxins have been used for decades as foliarly-applied biopesticides. However, these foliar applications are not routinely effective against CRW pests because the insect pests reside in the soil. Similar problems can be encountered with other, non-systemic, foliarly-applied chemical insecticides. The development and approval of transgenic corn plants expressing Bt delta-endotoxins active against coleopterans (e.g., modified Cry3A) should provide growers with another safe and efficacious option for the control of CRW.

Syngenta used recombinant DNA techniques to produce and introduce into corn, a restriction fragment containing the two transgenes: (1) the modified *cry3A* (*mcry3A*) gene encoding the mCry3A insect control protein and (2) the *pmi* (*manA*) gene from *Escherichia coli*, which encodes the enzyme phosphomannose isomerase (PMI) as a selectable marker. Expression of the *mcry3A* gene by corn plants renders the corn line resistant to CRW. Regulatory elements for the *mcry3A* and *pmi* genes were derived from maize and *Agrobacterium tumefaciens*. These regulatory sequences are not transcribed and do not encode proteins. In addition to transgenes necessary for insertion into the plant genome, the T-DNA vector also contained within the backbone two genes: (1) *Streptomycin* adenylyltransferase, *aadA,* gene from *E. coli*, conferring bacterial resistance to the antibiotics erythromycin, streptomycin, and spectinomycin and (2) consensus sequence for the origin of replication and partitioning region from plasmid pVS1 of *Pseudomonas*. The DNA was introduced into corn cells using *Agrobacterium*-mediated transformation methodology with the T-DNA transformation vector designated pZM26. Plant cells containing the introduced DNA were then selected by culturing in the presence of mannose. After the initial incubation with *Agrobacterium*, the broad-spectrum antibiotic cefotaxime was included in the culture medium to kill any remaining *Agrobacterium*. Because the transformed cells contain some sequences from a plant pest, they are explicitly subject to regulation under 7 CFR Part 340.

MIR604 corn has been field tested in the United States since 2001 as authorized by USDA notifications and permits listed in Table 1, on page 29 of the final revised petition. The list compiles a number of test sites in diverse regions of the U.S. including the major corn growing areas of the Midwest and winter nurseries in Hawaii and Puerto Rico. Field tests conducted under APHIS oversight allow for evaluation in a natural agricultural setting while imposing measures to minimize the risk of persistence in the environment after the completion of the test. Data are gathered on multiple parameters and are used by the applicants to evaluate agronomic characteristics and product performance and are used by APHIS to determine if the new variety poses a plant pest risk.

A. USDA Regulatory Authority

APHIS regulations at 7 CFR part 340, which were promulgated pursuant to authority granted by the Plant Protection Act (7 U.S.C. 7701-7772), regulate the introduction (importation, interstate movement, or release into the environment) of certain genetically engineered organisms and products. An organism is no longer subject to the regulatory requirements of 7 CFR Part 340 when it is demonstrated not to present a plant pest risk. A genetically engineered organism is considered a regulated article if the donor organism, recipient organism, vector or vector agent used in engineering the organism belongs to one of the taxa listed in the regulation and is also a plant pest, or if there is reason to believe that it is a plant pest. This corn has been considered a regulated article because it was genetically engineered with regulatory sequences derived from a bacterial plant pest.

Section 340.6 of the regulations, entitled "Petition for Determination of Nonregulated Status", provides that a person may petition the Agency to evaluate submitted data and determine that a particular regulated article does not present a plant pest risk, and therefore should no longer be regulated. If APHIS determines that the regulated article is unlikely to present a greater plant pest risk than the unmodified organism, the Agency can grant the petition in whole or in part. In such a case, APHIS authorizations (i.e., permits and notifications) would no longer be required for field testing, importation, or interstate movement of the non-regulated article or its progeny.

B. U.S. Environmental Protection Agency (EPA) and Food and Drug Administration (FDA) Regulatory Authority.

MIR604 corn 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, including herbicides, be registered before distribution or sale, unless exempted by EPA regulation. 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 practices, it will not cause unreasonable adverse effects on the environment.

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. The FDA enforces the tolerances set by the EPA. An exemption from the requirement of tolerance has been established for the PMI protein in all crops (69 *FR* 26770-26775). On October 27, 2004, the EPA announced two applications submitted by Syngenta: 1) a petition requesting an exemption from the requirement of a tolerance for residues of the mCRY3A protein and the genetic material necessary for their production in corn (69 FR 62688-62692) and 2) an application to register a pesticide product containing a new active ingredient (69 FR 62678-62680). On April 6, 2005, a temporary tolerance exemption was granted, exempting the requirement of a tolerance for residues of the mCRY3A protein and the genetic material necessary for their production in corn based on the conclusion that there was a reasonable certainty of no harm from consumption of the protein, as it is digestible in gastric fluid and not considered an allergen (70 *FR* 17323-17327). This temporary exemption was subsequently renewed (69 *FR* 11431-11433) and is currently set to expire on October 15, 2007 (71 *FR* 13269-13274). On January 25, 2006, the EPA announced the receipt of an application filed by Syngenta to amend an application for an Experimental Use Permit (EUP) to include the plant-incorporated protectant Event MIR604 mCry3A corn (71 *FR* 4141-4142). Also, on January 25, 2006, EPA announced Syngenta applied for an extension to the tolerance exemption expiring on October 15, 2006 (69 *FR* 11431-11433). The EPA held a meeting on March 14 and 15, 2006, of the Federal Insecticide, Fungicide, and Rodenticide Act Scientific Advisory Panel (FIFRA SAP) to consider and review human health and environmental issues associated with MIR604 Modified Cry3A Protein Bt Corn Plant Incorporated Protectant.

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. Under this policy, FDA uses what is termed a consultation process to ensure that human food and animal feed safety issues or other regulatory issues (e.g. labeling) are resolved prior to commercial distribution of a bioengineered food. Syngenta submitted a summary of their safety assessment on February 25, 2005, and additional information on March 21, 2006. The Syngenta assessment to the FDA indicated no changes in composition, safety or other relative parameters. The consultation for MIR604 corn as food and feed is currently underway.

III. Purpose and Need

APHIS prepared this EA before making a determination on the status of MIR604 corn as regulated articles under APHIS regulations. The developer of this corn, Syngenta, submitted a petition to USDA-APHIS requesting that APHIS make a determination that this corn shall no longer be considered a regulated article under 7 CFR Part 340. Under regulations in 7 CFR Part 340, APHIS is required to give a determination on the petition for non-regulated status. This EA was prepared in compliance with the National Environmental Policy Act (NEPA) of 1969 as amended, (42 U.S.C. 4321 *et seq.*) and the pursuant implementing regulations (40 CFR 1500-1508; 7 CFR Part 1b; 7 CFR Part 372).

IV. Alternatives

A. No Action: Continuation as a Regulated Article

Under the no action alternative, APHIS would come to a determination that MIR604 corn and its progeny should continue to be regulated under 7 CFR Part 340. Permits or acknowledgment of notifications from APHIS would still be required for their introduction. APHIS would choose this alternative if there were insufficient evidence to demonstrate lack of plant pest risk from the uncontained cultivation of MIR604 corn and its progeny.

B. Determination of Nonregulated Status

Under this alternative, MIR604 corn and its progeny would no longer be considered regulated articles under 7 CFR Part 340. Permits or notifications to APHIS would no longer be required for introductions in the United States and its territories of MIR604 corn or its progeny. A basis for this determination would be a finding that MIR604 is unlikely to pose a greater plant pest risk than the non-modified organism from which it was derived based on information submitted in the petition as stipulated in 7 CFR Part 340.6 (c) and other information that the Administrator believes to be relevant to a determination. Unrestricted cultivation of the lines would be permitted by APHIS. Such a determination, however, does not preclude any restriction on the cultivation of this corn that might be placed by other regulatory agencies also having authority.

C. Determination of Nonregulated Status, in Part

The regulations at 7 CFR Part 340.6 (d) (3) (i) state that APHIS may approve the petition in whole or in part. There are two ways in which a petition might be approved in part:

<u>Approval of some but not all lines requested in the petition</u>. In some petitions, applicants request that nonregulated status be granted to lines derived from more that one independent transformation event. In these cases, supporting data must be supplied for each line. APHIS could approve certain lines requested in the petition, but not others.

<u>Approval of the petition with geographic restrictions</u>. APHIS might determine that the regulated article poses no significant risk in certain geographic areas, but may pose a significant risk in others. In this case, APHIS may choose to approve the petition with a geographic limitation stipulating that the approved lines could only be grown in certain

geographic areas based on the identification of site-specific risks.

D. Preferred Alternative

APHIS has chosen Alternative B as the preferred alternative. This is based on the lack of plant pest characteristics in the MIR604 corn.

V. Affected Environment

A. Corn

Zea mays L. subsp. *mays* is a member of the *Maydeae* tribe of the grass family, *Poaceae*. It is a monoecious perennial plant that requires human intervention for its seed dispersal and propagation. The species is open-pollinated through wind movement of pollen. Additional information on the biology of maize can be found within the Organisation for Economic Co-Operation and Development (OECD) consensus document, which can be accessed at: http://www.oecd.org/LongAbstract/0,2546,en_2649_34385_8328413_119829_1_1_37437,00.html. Maize is primarily grown in the warm temperate climates (Norman *et al.* 1995) such as the 'Corn Belt' in the midwest United States, which consists of Iowa, Indiana, Illinois and Ohio as well as parts of South Dakota, Nebraska, Kansas, Minnesota, Wisconsin, Michigan, Missouri and Kentucky. The expression of the mCRY3A and the PMI proteins in the MIR604 corn line are not expected to alter the range of corn cultivation within the United States.

B. Corn Rootworm

Corn rootworms are the most serious insect pests in field corn in the U.S., costing growers millions of dollars each year in terms of insecticide use and crop loss (USDA-ARS, 2003).. Historically, crop rotation has provided effective protection from CRW damage. More recently, however, the effectiveness of crop rotation has become more limited because of several factors:

1. Many growers now prefer to grow corn continuously, as opposed to using crop rotation. Continuous corn production is a practice that necessitates higher inputs of chemical insecticides. The percentage of continuous corn acreage in the eastern and western Corn Belt states treated with insecticides ranges from 7%-100% (Gianessi *et al.*, 2002).

2. Crop rotation is not an effective management strategy for southern corn rootworm (SCRW) because it not only has a wide host range, but also because multiple generations can be produced in the same cornfield (Gianessi *et al.*, 2002). Larvae of SCRW can be found on the roots of corn, peanuts, alfalfa and cucurbits. There may be two to three generations of SCRW per year. Adults become active and lay eggs in the soil in late spring. These eggs hatch after one week and the larvae feed on corn roots for two to four weeks before pupating. A new generation of adults can emerge in mid-summer (Gianessi *et al.*, 2002).

3. A new NCRW biotype has exhibited extended diapauses in which some eggs can survive through a non-corn rotation to attack corn in a subsequent season (Ostlie, 1987; Tollefson, 1988; Gray *et al.*, 1998; Gianessi *et al.*, 2002). In South Dakota, Minnesota, Iowa, and Nebraska, the new NCRW biotype can diapause for two winters which allows the eggs to bypass the rotated crop and hatch in time to feed on the next corn crop (Gianessi *et al.*, 2002).

4. A new biotype of WCRW has appeared in central Illinois, northern Indiana and parts of Michigan that can lay eggs in soybean fields, so that the eggs hatch in the following season coinciding with the corn rotation (Onstad and Joselyn, 1999; O'Neal *et al.*, 1999; Gianessi *et al.*, 2002). This strain has spread rapidly since it was first observed in 1993, and it is expected to continue to spread throughout the Corn Belt.

As a result of these factors and the very damaging nature of the pest, the CRW complex is the most significant corn pest in the U.S. in terms of organophosphate chemical pesticide usage. The most common chemical regime is the application of a granular insecticide at planting, either banded or in-furrow. In some cases sprays are applied for adult suppression. Widespread use of chemical insecticides has raised concerns for worker safety, water contamination, and other environmental risks. Appendix B is a table comparing some of the most commonly used chemicals with respect to environmental fate and toxicity.

VI. Potential Environmental Impacts

Potential impacts to be addressed in this EA are those that pertain to the use of MIR604 corn and its progeny in the absence of confinement.

1. Potential impacts from gene introgression from MIR604 corn into its sexually compatible relatives.

In assessing the risk of gene introgression from MIR604 corn into its sexually compatible relatives, APHIS considers two primary issues: 1) the potential for gene flow and introgression; 2) the potential impact of introgression.

APHIS evaluated the potential for gene introgression to occur from MIR604 corn to sexually compatible wild relatives and considered whether such introgression would result in increased weediness. Cultivated corn, or maize, *Zea mays* L. subsp. *mays*, is sexually compatible with other members of the genus *Zea*, and to a much lesser degree with members of the genus *Tripsacum*.

In general, gene flow from cultivated agricultural crops to domesticated, wild or weedy relatives has most likely occurred ever since the domestication of a particular crop, assuming sexually compatible species are present (Stewart *et al.* 2003). Based upon currently available data, there have been a relatively low number of confirmed cases of introgression (Stewart *et al.* 2003).

Wild diploid and tetraploid members of *Zea* collectively referred to as teosinte are normally confined to the tropical and subtropical regions of Mexico, Guatemala, and Nicaragua; however, a fairly rare, sparsely dispersed feral population of teosinte has been reported in Florida. The Mexican and Central America teosinte populations primarily exist within and around cultivated maize fields; they are partially dependent on agricultural niches or open habitats, and in some cases are grazed upon or fed to cattle which distribute the seed. While some teosinte may be considered to be weeds in certain instances, they are also used by some farmers for breeding improved maize (Sánchez and Ruiz, 1997, and references therein). Teosinte is described to be susceptible to many of the same pests and diseases which attack cultivated corn (Sánchez and Ruiz, 1997).

All teosinte members can be crossed with cultivated corn to produce fertile F_1 hybrids (Doebley, 1990a; Wilkes, 1967). In areas of Mexico and Guatemala where teosinte and corn coexist, they have been reported to produce hybrids. Of the annual teosintes, *Z. mays* subsp. *mexicana* forms frequent hybrids with maize, *Z. luxurians* hybridizes only rarely with maize, whereas populations of *Z. mays* subsp. *parviglumis* are variable in this regard (Wilkes, 1977; Doebley, 1990a). Research on sympatric populations of maize and teosinte suggests introgression has occurred in the past, in particular from maize to *Z. mays* subsp. *luxurians* and *Z. mays* subsp. *diploperennis* and from annual Mexican plateau teosinte (*Z. mays* subsp. *mexicana*) to maize (Kato Y., 1997 and references therein).

Nonetheless, in the wild, introgressive hybridization from maize to teosinte is currently limited, in part, by several factors including distribution, differing degrees of genetic incompatibility, differences in flowering time in some cases, block inheritance, developmental morphology and timing of the reproductive structures, dissemination, and dormancy (Doebley, 1990a and 1990b; Galinat, 1988). First-generation hybrids are generally less fit for survival and dissemination in the wild, and show substantially reduced reproductive capacity which acts as a significant constraint on introgression. Teosinte has coexisted and co-evolved in close proximity to maize in the Americas over thousands of years, but maize and teosinte maintain distinct genetic constitutions despite sporadic introgression (Doebley, 1990a). The potential for gene introgression from MIR604 corn into teosinte would increase if varieties are developed, and approved for cultivation in locations where these teosintes are located. A limited potential can also occur through smuggling unapproved seeds or from import grain for planting. Since MIR604 corn does not exhibit characteristics that cause it to be any more weedy than other cultivated corn, its potential impact due to the limited potential for gene introgression into teosinte is not expected to be any different from that of other cultivated maize varieties.

The genus *Tripsacum* contains up to 16 recognized species, most of which are native to Mexico, Central and South America, but three of which exist as wild and/or cultivated species in the U.S. Though many of these species occur where corn might be cultivated, gene introgression from MIR604 corn under natural conditions is highly unlikely or impossible. Hybrids of *Tripsacum* species with *Zea* are difficult to obtain outside of a laboratory and are often sterile or have greatly reduced fertility, and none are able to withstand even the mildest winters. Furthermore, none of the sexually compatible relatives of corn in the U.S. are considered to be weeds in the U.S. (Holm *et al.*, 1979), therefore, the unlikely acquisition of a single pesticide gene or the *pmi* gene would not be expected to transform them into weeds.

2. Potential impacts based on the relative weediness of MIR604 corn

APHIS assessed whether MIR604 corn is any more likely to become a weed than the nontransgenic recipient corn line, or other corn currently cultivated. The assessment encompasses a thorough consideration of the basic biology of corn and an evaluation of unique characteristics of MIR604 corn.

In the U.S., 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). Furthermore, corn has been grown throughout the world without any report that it is a serious weed. Cultivated corn is unlikely to become a weed. It is not generally persistent in undisturbed environments without human intervention. Although corn volunteers are not uncommon, they are easily controlled by herbicides or mechanical means. Corn also possesses few of the characteristics of plants that are notably successful weeds (Baker, 1965; Keeler, 1989).

Syngenta conducted agronomic field trials at a total of 32 field trial locations in the U.S. Corn Belt during the 2002 and 2003 growing seasons. Table 4 (revised petition, page 58) identifies the traits assessed in the Agronomic Field Trials. For the majority of the traits assessed, there were no statistically significant differences between MIR604-derived hybrids and their negative segregant control counterparts. There were few statistically significant differences between the MIR604-derived hybrids and their negative segregant controls, as identified in Appendix 1C, Tables 1C to 4C and Appendix 1D, Tables 2D to 4D of the revised petition. Most of these differences were not consistent at the different sites over the two years of field trials. For example, one or both of the MIR604-hybrids exhibited a 'grain moisture percentage measured at harvest time' (GMSTP) that was significantly lower in the MIR604-derived hybrids at 9 field trial locations, significantly higher at 1 field trial location, and exhibited no difference at 10 of the field trials locations. At these 32 locations, the range of values for agronomic parameters, even when significantly different, was within the range of values expected for traditional maize hybrids. The results of these field trials indicate that MIR604 corn does not exhibit characteristics that would cause it to be more weedy than the parental corn line.

In addition, Syngenta conducted disease trials in 2002 and 2003, whereby MIR604-hybrids and their negative segregant controls were exposed to various corn pathogens, including Northern corn leaf blight (*Helminthosporium turcicum*), Southern corn leaf blight (*Helminthosporium maydis*), Eyespot (*Kabatiella zeae*), and Gray leaf spot (*Cercospora zeae-maydis*). Lesion density and spread were measured. No significant differences in disease susceptibility were found between line MIR604 corn and the non-transgenic counterparts (revised petition, Appendix 1C, Table 6C). The results of these trials indicate that MIR604 corn does not exhibit characteristics that would cause it to be more susceptible to disease than the parental corn line.

The introduced traits, coleopteran insect resistance and mannose utilization, are not expected to cause MIR604 corn to become a weed. Other CRW-resistant corn varieties previously deregulated by APHIS did not exhibit characteristics that would enhance weediness (APHIS assessments are available at: http://www.aphis.usda.gov/brs/not_reg.html). None of the characteristics of weeds described by Baker (1965) involve resistance or susceptibility to insects, and there is no reason to expect that the protection against the target insects provided by this new corn line would release it from any constraint that would result in increased weediness. MIR604 corn is still susceptible to other insect pests and diseases of corn and it is unchanged in its susceptibility to injury by commercially available herbicides.

3. Potential impact on non-target organisms, including beneficial organisms and threatened or endangered species

APHIS evaluated the potential for line MIR604 corn plants and their products to have damaging or toxic effects directly or indirectly on non-target organisms. Non-target organisms considered were those representative of the exposed agricultural environment, including those that are recognized as beneficial to agriculture or as threatened or endangered in the U.S. APHIS also considered potential impacts on other "non-target" pests, since such impacts could potentially change agricultural practices.

The *pmi* (*manA*) gene comes from *E. coli* and encodes the enzyme phosphomannose isomerase (PMI). *Pmi* serves as a marker gene that enables selection of Bt lines, providing the plant with the ability to utilize mannose as a sole carbon source. The expression of PMI protein in corn plants is not expected to have deleterious effects or significant impacts on non-target organisms, including beneficial organisms, based on data provided in the petition. Additionally, the EPA has granted an exemption from the requirement of a tolerance for the PMI protein as an inert ingredient in all plants (U.S. EPA 2004a). The DNA encoding the PMI protein is not toxic. At the 80-amino acid peptide level, the PMI protein shares no significant homology with proteins known to be toxic or allergenic. Within one of the 80-amino acid windows, there was one region of sequence homology of eight contiguous amino acids between MIR604 PMI and a recently described allergen, α-parvalbumin, from *Rana* species (frog). Further testing found no cross-reactivity between the human serum Immunoglobulin E (IgE) and Bovine Serum Albumin (BSA), indicating that the low degree of sequence identity between MIR604 PMI and α-parvalbumin from *Rana* species is not biologically relevant.

Like the Cry1 class of insecticidal proteins, the specificity of the mCry3A protein insecticidal activity is dependent upon their binding to specific receptors present in the insect mid-gut (Lambert, *et al.*, 1996; Van Rie *et al.*, 1990; Van Rie *et al.*, 1989; Hofmann *et al.*, 1988a and 1988b; and Wolfersberger *et al.*, 1986). These insecticidal proteins are not expected to adversely affect other invertebrates or vertebrate organisms, including non-target birds, mammals and humans. APHIS evaluated laboratory and field studies on representative species that support these expectations. The toxicity and specificity of the coleopteran specific Cry proteins is associated with their solubilization and proteolytic activation in the insect midgut, and their binding to specific cell membrane receptors in the brush border membrane vesicles present in the midgut of susceptible insects. These specific receptors are not expected to be present in non-target birds, mammals, and humans (Griffitts *et al.*, 2005;

Lambert *et al.*, 1996; Van Rie *et al.*, 1990; Van Rie *et al.*, 1989; Hofmann *et al.*, 1988a and 1988b; and Wolfersberger *et al.*, 1986).

Potential impacts on target and non-target pests:

The mCry3A protein only has enhanced activity over the native Cry3A protein against select beetle (Order: Coleoptera) species within the family Chrysomelidae, namely corn rootworm. Syngenta conducted a series of diet bioassays with microbially-expressed mCry3A proteins to characterize the insecticidal specificity (see revised petition Chapter 7, Table 14, page 75). Test species included the target Coleopteran species: Northern corn rootworm (*D. barberi*), Western corn rootworm (*D. virgifera virgifera*), Southern corn rootworm (aka spotted cucumber beetle, *D. undecimpunctata howardi*); non-target Coleopteran pests: Colorado potato beetle (CPB; *Leptinotarsa decemlineata*), banded cucumber beetle (*Diaborotica balteata*) and cotton boll weevil (*Anthonoms grandis*). Additionally, test species also included lepidopteran pests, including: black cutworm (*Agrotis ipsilon*), corn earworm (*Heliocoverpa zea*), European corn borer (*Ostrinia nubilalis*), fall armyworm (*Spodoptera frugiperda*), pink bollworm (*Pectinophora gossypiella*) and tobacco budworm (*Heliothis virescens*). The microbially-expressed mCry3A protein exhibited activity against the following Coleopterans: CPB, Western corn rootworm, Northern corn rootworm, and banded cucumber beetle. For all but CPB, against which both the native Cry3A and mCry3A proteins were active, the mCry3A exhibited enhanced activity over the native Cry3A. However, neither the native nor mCry3A was active against the lepidopteran pests tested. Field trials of MIR604 corn plants also verified that corn plants expressing the mCry3A were better protected against NCRW and WCRW than nontransgenic corn plants (see revised petition Appendix 1A, Tables 2A and 3A) and MCRW (revised petition Appendix 1A, Table 5A). An additional glasshouse trial verified that MIR604-derived hybrids were better protected against NCRW than nontransgenic control hybrids (revised petition Appendix 1A, Table 4A).

Potential impacts on non-target organisms, including beneficial organisms:

The mCry3A protein is not expected to adversely affect non-target invertebrate and vertebrate organisms, including birds, mammals and humans, because they are not expected to contain the receptor found in the midgut of target insects. To evaluate the potential of line MIR604 corn to have damaging or toxic effects on representative terrestrial and an aquatic species, APHIS assessed data from a series of ecological toxicology experiments including the results of several studies submitted that were designed to evaluate the sensitivity of representative non-target organisms to mCry3A protein. Test substrates included corn plant material (e.g., corn grain, leaf or pollen) expressing mCry3A protein or protein purified from *E. coli* strain DH5α engineered to express the mCry3A protein. Syngenta verified that the bacterially-produced mCry3A protein, as purified and prepared for these studies, was similar in its biochemical properties (molecular weight, amino acid sequence and lack of glycosylation) and in biological activity against WCRW and thus was relevant to use as a test substance comparable to mCry3A as produced in line MIR604 corn.

Acute dietary toxicity studies of beneficial arthropods were conducted in laboratory tests and no adverse effects were observed at levels 10.6 to 36 times the estimated environmental exposure (EEC) calculated using estimates of corn consumption for each organism (revised petition, Table 19, page 87). MIR604 pollen does not contain detectable levels of mCry3A protein and therefore pollinators, like honey bees (*Apis mellifera*), will be exposed to negligible amounts of mCry3A. However, standard test methods exist for larval *A. mellifera*, whereby deleterious effects of the test substance may be evaluated at a sensitive developmental stage. Beneficial natural enemies, insidious flower bug (aka minute pirate bug; *Orius insidiosus*) and the seven spotted lady beetle (*Coccinella septempunctata*) were fed microbially-produced protein mixed with artificial diet as well as representative ground-dwelling predators, a rove beetle species (*Aleochara bilineata*) and a ground beetle species (*Poecilus cupreus*). Since parasitic and predatory insects will have limited direct exposure to the mCry3A insecticidal protein expressed in line MIR604 corn, little impact is expected for these species other than a possible shift to alternate hosts since corn rootworm populations are expected to be reduced.

The sensitivity of other organisms to mCry3A was tested using microbially-expressed mCry3A protein. The additional organisms tested included earthworms (*Eisenia foetida*) as a representative decomposer, rainbow trout (*Oncorhynchus mykiss*), and bobwhite quail (*Colinus virginianus*). All of the organisms evaluated in the dietary toxicity studies were exposed to much greater levels of the mCry3A proteins than they would be exposed in the field (see revised petition, Chapter 7. Environmental Safety of mCry3A; Table 19, page 87) with no adverse effects observed.

In Chapter 9, Environmental Consequences of Introduction, the petitioner estimates that with the availability of MIR604 corn on the market, there could be a substantial reduction in the use of conventional pesticides, citing the potential elimination of 4.5 million acre pesticide treatments and approximately 1.25 million pounds of active ingredients within the first five years in sales of MIR604 corn. Tables 22 and 23 identify the corn rootworm pesticides by class currently used, including amount used (estimated pounds and percentage of use), acreage treated and grower cost. In general, mCry3A protein expressed in corn line MIR604 compares favorably to these products with respect to the reduced potential for harm in the environment.

Potential impacts on threatened and endangered arthropods:

APHIS coordinates review of petitions with other agencies that have regulatory oversight on these same products. With respect to threatened and endangered species, EPA also plays a role in the evaluation. Given the specificity of the mCry3A activity, species outside the insect order Coleoptera and Chrysomelidae family should not be affected. There are no endangered Chrysomelidae within the U.S. APHIS has thoroughly examined all threatened and endangered coleopterans that occur in counties where corn is grown, and determined that the breeding habitat of coloepterans does put them in proximity to corn fields.

Based on a lack of exposure, no unreasonable adverse effects of mCry3A corn to endangered coleoptera are expected. Many of the endangered and threatened beetles occur in cave or aquatic habitats. None of the endangered beetles are expected to occur in or near cornfields. The American burying beetle (*Nicrophorus americanus*) may occur in old fields or cropland hedge rows. However, based upon the feeding habits of the American burying beetle, it is not expected to occur within cornfields nor will it be exposed to mCry3A protein. Adult American burying beetles are classified as opportunistic scavengers that feed on anything dead and bury vertebrate carcasses which larvae feed on. Larvae is fed carrion that is regurgitated by adults until the larvae are able to feed directly on a carcass.

BRS has reviewed the data in accordance with a process mutually agreed upon with the U.S. Fish and Wildlife Service (FWS) to determine when a consultation, as required under section 7 of the Endangered Species Act, is needed. APHIS has reached a determination that the release following a determination of nonregulated status would have no effects on listed threatened or endangered species or their critical habitat and consequently, consultation with FWS is not required for this EA.

Environmental fate in soil:

An insect bioassay was conducted in the laboratory with the CPB to determine the DT_{50} (time to 50% degradation) of the mCry3A protein in soil. Mortality of the CPB was monitored and modeled using first-order kinetics to determine the DT_{50}. The laboratory bioassay established a DT_{50} of 7.6 days.

4. Potential impacts on biodiversity

Our analysis concludes that line MIR604 corn exhibits no traits that would cause increased weediness, that its unconfined cultivation should not lead to increased weediness of other cultivated corn or other sexually compatible relatives, and it is unlikely to harm non-target organisms common to the agricultural ecosystem or threatened or endangered species recognized by the U.S. Fish and Wildlife Service. Based on this analysis, there is no apparent potential for significant impact to biodiversity. If APHIS chooses the no action alternative, there would also be no impact on biodiversity.

5. Potential impacts on agricultural and cultivation practices

APHIS considered potential impacts associated with the cultivation of rootworm-resistant corn line MIR604 on current agricultural practices, in particular, those used to control CRW in corn. The potential impact on organic farming was also considered.

Potential impacts of line MIR604 corn on insect control practices

Syngenta has provided data which indicate that MIR604 corn expresses the mCry3A protein in root tissues to provide control of corn rootworms. The availability of this product is likely to have an impact on current control practices for corn rootworm that include the use of crop rotation, chemical insecticides, and other Bt corn varieties that are intended to control corn rootworm. Both crop rotation and the use of chemical insecticides have been important strategies in the past. However, CRW have developed several adaptations to control methods including crop rotation and insecticide resistance. Since CRW predominantly oviposit in cornfields, rotating corn with small grains, hay, clover or alfalfa has been utilized as a control method (Levine and Oloumi-Sadeghi 1991).

Soybean rotations was formerly an effective strategy to control CRW in corn and thereby minimize pesticide application. However, WCRW has developed an adaptation to resist the corn/soybean rotation in Illinois and Indiana (Levine and Oloumi-Sadeghi 1996). In areas such as east-central Illinois and northern Indiana, the WCRW has been found to have the ability to lay eggs in soybean, overwinter and hatch the following year in corn (Levine and Oloumi-Sadeghi 1991, Levine and Oloumi-Sadeghi 1996, O'Neal *et al.* 1999, Isard *et al.* 1999, Isard *et al.* 2000). Northern CRW populations have also developed resistance to the corn/soybean rotation in Minnesota, Iowa, and South Dakota (Gray *et al.* 1998). Prolonged diapause of NCRW involves eggs that remain viable for two winters and hatch two seasons after being laid. Northern CRW have developed the ability for prolonged or extended diapause resulting in a significant proportion of their eggs hatching after two winters leading to an adaptation to rotating corn with crops such as soybean. Extended diapause has been verified in the laboratory from NCRW eggs collected from South Dakota, Minnesota, Illinois and Michigan (Krysan *et al.* 1984, Krysan *et al* 1986, Levine and Oloumi-Sadeghi 1991 Levine *et al.* 1992a, Levine *et al.* 1992b). Field studies conducted by Tollefson (1988) in northwestern Iowa cornfields suggest that extended diapause occurs throughout NCRW distribution in rotated fields. Another study conducted by Levine and Oloumi-Sadeghi (1996) suggests that the WCRW does not demonstrate extended diapause. In these cases, resistance took at least ten and usually more than 15 years to develop without implementing insect resistance management (IRM) strategies. Instances of CRW resistance to crop rotation and/or insecticide use typically develop on a local scale which is probably due to limited adult movement before and after mating. Research is currently underway at the University of Nebraska and USDA-ARS in North Dakota to determine the genetics of esterase-mediated insecticide resistance in WCRW populations. Results of this research are intended to provide knowledge on localized selection and migration that may aid in refining future IRM strategies.

In addition to the problem with insect adaptation to crop rotation, many growers simply prefer to grow corn continuously, a practice which necessitates higher inputs of chemical insecticides. In 2001, about 18% of all corn acres were treated for CRW with insecticides. However, producers growing continuous corn had a much higher incidence of soil insecticide use; with about 38% of these acreas treated with insecticides for CRW (Payne et al. 2003).

With crop rotation losing its effectiveness to provide adequate CRW control, the primary alternative to insect-resistant GE corn is traditional insecticide use. More than nine million pounds of insecticide were applied to the 2001 US corn crop (Payne et al. 2003). The most widely used insecticides are from the organophosphate or synthetic pyrethroid classes of chemistry. It is therefore expected that availability of another practical and economical alternative to chemical insecticides for CRW control would result in a significant reduction in application of such chemicals.

The EPA has produced a number of documents regarding the use of Bt technology in corn. A risks and benefits assessment for reregistration of Bt corn and cotton plant incorporated protectants (PIP's) has been prepared by the EPA (U.S. EPA, 2000) and is posted at the following EPA internet site: http://www.epa.gov/scipoly/sap/meetings/2000/index.htm. Issues considered by the EPA pertaining to this assessment were the subject of a meeting convened on October 18-20, 2000, by the EPA Federal Scientific Advisory Panel (SAP). In 2001, EPA issued a registration document for *Bacillus thurigiensis* Plant-incorporated protectants. In this document, EPA confirms their original findings that "there are no unreasonable adverse health effects from these products" and that there are no unreasonable adverse effects in corn on nontarget wildlife or beneficial organisms (US EPA, 2001). EPA also convened a SAP meeting, August 27-29, 2002, to consider issues related to corn rootworm-related PIP's. The results of this SAP meeting can be found at: http://www.epa.gov/scipoly/sap/2002/index.htm. An SAP was also held for MIR604 corn on March 14-15, 2006 (http://www.epa.gov/scipoly/sap/meetings/2006/march/finalmeetingminutes6_1_2006.pdf).

Before these new Bt corn varieties were available, farmers were willing to accept lower corn yields, rather than incur the expense, trouble, and uncertain results of chemical insecticide applications to control the target pests. With Bt seed technology, each individual plant is protected, resulting in reduced insecticide use where insecticides are used to control for CRW, lower labor costs and increased yields during significant CRW infestation relative to non-Bt fields (Payne et al. 2003). Following the registration of Bt corn varieties in 1995, growers were quick to embrace the new technology. Estimates of Bt corn acreage as a percent of total corn acreage planted increased from 1% in 1996 to 40%*[*] in 2006 (USDA NASS summarized at http://www.ers.usda.gov/data/biotechcrops/ExtentofAdoptionTable1.htm).

MIR604 corn could be incorporated into current integrated pest management (IPM) practices as an additional tool for control. Fields are typically scouted for adult CRW in the late summer or early fall. Economic thresholds are then used in making decisions about control strategies for the following spring planting season. MIR604 offers an alternative to organophosphate and pyrethroid insecticide applications in cases where thresholds indicate CRW control is needed and the grower chooses to grow corn. No new or specialized equipment or skills would be needed to use the new technology. Reduced pesticide usage by the growers would carry the accompanied benefits of reduced needs for the manufacture, transport, storage and disposal of hazardous chemicals and containers.

In order to delay the potential evolution of resistance in the target pests to Bt Cry proteins expressed in plants, growers have been required by the EPA and/or the developers to implement insect resistance management (IRM) strategies. Syngenta has submitted to EPA, a detailed strategy for approval prior to commercialization of this product. The plan includes monitoring for compliance with the IRM plant, grower education, monitoring for resistance

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[*] 40% value is the sum of insect resistant (Bt) corn and stacked varieties (varieties with herbicide tolerant and insect resistant traits)

to development of resistant CRW populations and mitigation measures if resistant populations are confirmed. Such insect management strategies may be responsible, in part, for delaying the development of resistance to the Cry toxins. Cry3Bb1 corn has been registered by EPA for commercial production since 2002 and there have been no reports of coleopteran insect resistance developing in the field to any Bt toxin expressed in any plant

Potential impacts of line MIR604 corn on weed control

APHIS evaluated data submitted by the petitioner that show that hybrids derived from line MIR604 corn express mCry3A. Line MIR604 corn is expected to have no impact on current agricultural practices used for weed control as it is no more herbicide tolerant than its nonengineered counterpart.

Volunteers of line MIR604 corn can be controlled by selective mechanical or manual weed removal or by the use of several commercially available herbicides. For example, in soybean, which is the crop most commonly rotated with corn, herbicides based on sulfonylurea, lipid biosynthesis inhibitors, or Fluazifop/fomesafen could be used to control maize volunteers. The commercial introduction and wide adoption in the United States of Roundup Ready® soybeans has been associated with an increase in the use of glyphosate to control weeds in soybean, while the use of other herbicides has decreased (Fernandez-Cornejo and McBride, 2000; Heimlich *et al.*, 2000). Glyphosate could also be used to control volunteers of line MIR604 corn in Roundup Ready® soybeans. It is estimated that in 1996, 7% of the total soybean acreage was planted to herbicide tolerant soybeans, compared to an estimated 82% of total soybean acreage planted to herbicide tolerant soybeans in 2003 (Sankula and Blumenthal, 2004). Additionally, glufosinate could be used. Both glyphosate and glufosinate have relatively low toxicity to humans and wildlife, and do not persist in the environment (Pike, 1999; McGlamery *et al.* 1999).

Potential impacts on organic farming

The National Organic Program (NOP) is administered by USDA's Agricultural Marketing Service (AMS). Organic production operations must develop and maintain an organic production system plan approved by their accredited certifying agent in order to obtain certification. Organic certification of a production or handling operation is a process claim, not a product claim. Organic certification involves oversight by an accredited certifying agent of the materials and practices used to produce or handle an organic agricultural product. Oversight by a certifying agent includes an annual review of the certified operation's organic system plan and on-site inspections of the certified operation and its records.

The organic system plan enables the production operation to achieve and document compliance with the National Organic Standards, including the prohibition on the use of excluded methods. Excluded methods include a variety of methods used to genetically modify organisms or influence their growth and development by means that are not possible under natural conditions or processes. Although the National Organic Standards prohibit the use of excluded methods, they do not require testing of inputs or products for the presence of excluded methods, unless a certifying agent has reasonable suspicion that a prohibited substance or excluded method was used. The presence of a detectable residue of a product of excluded methods alone does not necessarily constitute a violation of the National Organic Standards.

It is not likely that organic farmers, or other farmers who choose not to plant transgenic varieties or sell transgenic grain, will be significantly impacted by the expected commercial use of this product since: (a) nontransgenic corn will likely still be sold and will be readily available to those who wish to plant it; (b) farmers purchasing seed will know this product is transgenic because it will be marketed as *Bt mCry3A* coleopteran resistant; and (c) based on the IRM plan, farmers will be educated about recommended management practices. Transgenic corn lines resistant to coleopteran insects, and/or tolerant to glufosinate are already in widespread use by farmers. This particular product should not present new and different issues than those with respect to impacts on organic farmers. APHIS has considered that corn is open-pollinating and it is possible that the engineered genes could move via wind-blown pollen to an adjacent field. All corn, whether genetically engineered or not, can transmit pollen to nearby fields, and a very small influx of pollen originating from a given corn variety does not appreciably change the characteristics of corn in adjacent fields. As described previously in this assessment, the rate of cross-pollination from one field to another is expected to be quite low, even if flowering times coincide. The frequency of such an occurrence decreases with increasing distance from the pollen source such that it sufficiently low at 660 feet away to be considered adequate for production of certified corn seeds. Methods are currently available to prevent or minimize and test for cross-contamination.

6. Potential impacts on raw or processed agricultural commodities.

APHIS analysis of data on agronomic performance, disease and insect susceptibility, and compositional profiles of the kernels indicate no differences between MIR 604 and their non-transgenic hybrid counterparts that would be expected to cause either a direct or indirect plant pest effect on any raw or processed plant commodity from deregulation of line MIR604.

VII. Consideration of Executive Orders, Standards and Treaties Relating to Environmental Impacts

Executive Order (EO) 12898, "Federal Actions to Address Environmental Justice in Minority Populations and Low-Income Populations," requires Federal agencies to conduct their programs, policies, and activities that substantially affect human health or the environment in a manner so as not to exclude persons and populations from participation in or benefiting from such programs. It also enforces existing statutes to prevent minority and low-income communities from being subjected to disproportionately high and adverse human health or environmental effects.

EO 13045, “Protection of Children from Environmental Health Risks and Safety Risks,” acknowledges that children may suffer disproportionately from environmental health and safety risks because of their developmental stage, greater metabolic activity levels, and behavior patterns, as compared to adults. The EO (to the extent permitted by law and consistent with the agency's mission) requires each Federal agency to identify, assess, and address environmental health risks and safety risks that may disproportionately affect children.

Each alternative was analyzed with respect to EO 12898 and 13045. None of the alternatives are expected to have a disproportionate adverse effect on minorities, low-income populations, or children. Collectively, the available mammalian toxicity, along with the history of safe use of microbial Bt products and other corn varieties expressing Bt proteins, establishes the safety of corn line MIR604 and its products to humans, including minorities, low income populations, and children who might be exposed to them through agricultural production and/or processing. No additional safety precautions would need to be taken. None of the impacts on agricultural practices expected to be associated with deregulation of corn line MIR604 described above are expected to have a disproportionate adverse effect on minorities, low income populations, or children. As noted above, the cultivation of previously deregulated corn varieties with similar insect resistance traits has been associated with a decrease and/or shift in pesticide applications for those who adopt these varieties that is either favorable or neutral with respect to environmental and human toxicity. If pesticide applications are reduced, there may be a beneficial effect on children and low income populations that might be exposed to the chemicals. These populations might include migrant farm workers and their families, and other rural-dwelling individuals who are exposed to pesticides through ground-water contamination or other means of exposure. It is expected that EPA and USDA Economic Research Service would monitor the use of this product to determine impacts on agricultural practices such as chemical use as they have done previously for Bt products.

EO 13112, "Invasive Species", states that Federal agencies take action to prevent the introduction of invasive species and provide for their control and to minimize the economic, ecological, and human health impacts that invasive species cause. Nonengineered corn as well as other Bt and herbicide tolerant corn varieties are widely grown in the U.S. Based on historical experience with these varieties and the data submitted by the applicant and reviewed by APHIS, the engineered plant is sufficiently similar in fitness characteristics to other corn varieties currently grown, and it is not expected to have an increased invasive potential.

Executive Order 12114, "Environmental Effects Abroad of Major Federal Actions" requires Federal officials to take into consideration any potential environmental effects outside the U.S., its territories and possessions that result from actions being taken. APHIS has given this due consideration and does not expect a significant environmental impact outside the U.S. should nonregulated status be determined for corn line MIR604 or if the other alternatives are chosen. It should be noted that all the considerable, existing national and international regulatory authorities and phytosanitary regimes that currently apply to introductions of new corn cultivars internationally, apply equally to those covered by an APHIS determination of nonregulated status under 7 CFR Part 340. Any international traffic in MIR604 corn subsequent to a determination of non-regulated status for line MIR604 would be fully subject to national phytosanitary requirements and be in accordance with phytosanitary standards developed under the International Plant Protection Convention (IPPC).

The purpose of the IPPC “is to secure a common and effective action to prevent the spread and introduction of pests of plants and plant products, and to promote appropriate measures for their control” (http://www.ippc.int/IPP/En/default.htm). The protection it affords extends to natural flora and plant products and includes both direct and indirect damage by pests, including weeds. The IPPC has set a standard for the reciprocal acceptance of phytosanitary certification among the nations that have signed or acceded to the Convention (116 countries as of June, 2001). In April, 2004, a standard for pest risk analysis of living modified organisms (LMOs) was adopted at a meeting of the governing body of the IPPC as a supplement to an existing standard, International Standard for Phytosanitary Measure No. 11 (ISPM-11; Pest Risk Analysis for Quarantine Pests). The standard acknowledges that all LMOs will not present a pest risk, and that a determination needs to be made early in the PRA for importation as to whether the LMO poses a potential pest risk resulting from the genetic modification. APHIS pest risk assessment procedures for bioengineered organisms are consistent with the guidance developed under the IPPC. In addition, issues that may relate to commercialization and transboundary movement of particular agricultural commodities produced through biotechnology are being addressed in other international forums and through national regulations.

The Cartagena Protocol on Biosafety is a treaty under the United Nations Convention on Biological Diversity (CBD) that established a framework for the safe transboundary movement, with respect to the environment and biodiversity, of LMOs, which includes those modified through biotechnology. The Protocol came into force on September 11, 2003 and 119 countries are parties to it as of April 14, 2005 (see http://www.biodiv.org/biosafety/default.aspx). Although the U.S. is not a party to the CBD, and thus not a party to the Cartagena Protocol on Biosafety, U.S. exporters will still need to comply with domestic regulations that importing countries that are parties to the Protocol have put in place to comply with their obligations. The first intentional transboundary movement of LMOs intended for environmental release (field trials or commercial planting) will require consent from the importing country under an advanced informed agreement (AIA) provision, which includes a requirement for a risk assessment consistent with Annex III of the Protocol, and the required documentation. LMOs imported for food, feed or processing (FFP) are exempt from the AIA procedure, and are covered under Article 11 and Annex II of the Protocol. Under Article 11 Parties must post decisions to the Biosafety Clearinghouse database on domestic use of LMOs for FFP that may be subject to transboundary movement. To facilitate compliance with obligations to this protocol, the US Government has developed a website that provides the status of all regulatory reviews completed for different uses of bioengineered products (http://usbiotechreg.nbii.gov). These data will be available to the Biosafety Clearinghouse.

APHIS continues to work toward harmonization of biosafety and biotechnology consensus documents, guidelines and regulations, including within the North American Plant Protection Organization (NAPPO), which includes Mexico, Canada, and the U.S. and in the Organization for Economic Cooperation and Development (OECD). NAPPO has completed three modules of a standard for the *Importation and Release into the Environment of Transgenic Plants in NAPPO Member Countries* (see http://www.nappo.org/Standards/Std-e.html). APHIS also participates in the North American Biotechnology Initiative (NABI), a forum for information exchange and cooperation on agricultural biotechnology issues for the U.S., Mexico and Canada. In addition, bilateral discussions on biotechnology regulatory issues are held regularly with other countries including: Argentina, Brazil, Japan, China, and Korea. Many countries, e.g. Argentina, Australia, Canada, China, Japan, Korea, Philippines, South Africa, Switzerland, the United Kingdom, and the European Union have already approved Bt corn varieties to be grown or imported for food or feed (http://www.agbios.com/dbase.php).

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Appendix A. Potential for introgression from *Zea mays* to its sexually compatible relatives.

Wild diploid and tetraploid members of *Zea* collectively referred to as teosinte are normally confined to the tropical and subtropical regions of Mexico, Guatemala, and Nicaragua. A few isolated populations of annual and perennial teosinte have been reported to exist in Florida and Texas, respectively, but local botanists and agronomists familiar with the flora of these regions have not documented any current populations of teosinte (U.S. EPA, 2000). The Mexican and Central America teosinte populations primarily exist within and around cultivated maize fields; they are partially dependent on agricultural niches or open habitats, and in some cases are grazed upon or fed to cattle which distribute the seed. While some teosinte may be considered to be weeds in certain instances, they are also used by some farmers for breeding improved maize (Sánchez and Ruiz, 1997, and references therein).

All teosinte members can be crossed with cultivated corn to produce fertile F_1 hybrids (Doebley, 1990a; Wilkes, 1967; and Jesus Sánchez, personal communication, 1998). In areas of Mexico and Guatemala where teosinte and corn coexist, they have been reported to produce hybrids. Of the annual teosinte, *Z. mays* ssp *mexicana* forms frequent hybrids with maize, *Z. luxurians* hybridizes only rarely with maize, whereas populations of *Z. mays* ssp. *parviglumis* are variable in this regard (Wilkes, 1977; Doebley, 1990a). Fewer fertile hybrids are found between maize and the perennial *Z. perennis* than are found with *Z. diploperennis* (J. Sánchez, personal communication, 1998). Research on sympatric populations of maize and teosinte suggests introgression has occurred in the past, in particular from maize to *Z. mays* ssp. *luxurians* and *Z. mays* ssp. *diploperennis* and from annual Mexican plateau teosinte (*Z. mays* ssp. *mexicana*) to maize (Kato, 1997 and references therein). Nonetheless, in the wild, introgressive hybridization from maize to teosinte is currently limited, in part, by several factors including distribution, differing degrees of genetic incompatibility, differences in flowering time in some cases, block inheritance, developmental morphology and timing of the reproductive structures, dissemination, and dormancy (Doebley, 1990a; Galinat, 1988). First-generation hybrids are generally less fit for survival and dissemination in the wild, and show substantially reduced reproductive capacity which acts as a significant constraint on introgression. Teosinte has coexisted and co-evolved in close proximity to maize in the Americas over thousands of years, but maize and teosinte maintain distinct genetic constitutions despite sporadic introgression (Doebley, 1990a).

The genus *Tripsacum* contains up to 16 recognized species, most of which are native to Mexico, Central and South America. But three *Tripsacum* species, *T. floridanum*, *T. lanceolatium*, and *T. dactyloides*, exist as wild and/or cultivated in the U.S. (Hitchcock, 1971). Though many of these species occur where corn might be cultivated, gene introgression from line 1507 corn under natural conditions is highly unlikely or impossible. Hybrids of *Tripsacum* species with *Zea* are difficult to obtain outside of a laboratory and are often sterile or have greatly reduced fertility, and none are able to withstand even the mildest winters (Beadle, 1980; Galinat, 1988).

References (see EA, Literature Cited, Section VII.)

Appendix B. **Environmental and human health safety of mCry3A (as expressed in corn line MIR604 or as purified from a microbial source) compared to other common insecticides used on corn to control the corn rootworm target pests, and other non-target pests.**

	mCry3A	Terbufos (Counter®)	Tefluthrin (Force®)
Environmental Fate	The DT_{50} estimate for mCry3A protein in soil was found to be 7.6 days. (1)	Terbufos hydrolyzes at pH 5, 7, and 9 with a half-life of 2.2 weeks. Formaldehyde was the major degradate detected in this study. Aerobic soil metabolism study indicate that terbufos degrades in silt loam soil with a half-life of 26.7 days. The major degradates detected in this study included carbon dioxide, terbufos sulfoxide, and terbufos sulfone. Terbufos residues have a half-life of less than 40 days in field plots of loam soil treated with a 15 percent granular formulation at an application rate of 1 lb ai/A. The sampling protocol was inadequate to accurately assess the dissipation of terbufos residues in field soil and a new study is required. The available data reviewed by the Agency are not sufficient to fulfill data requirements nor to assess the environmental fate of terbufos. EPA is concerned about the potential for the two degradates, terbufos sulfoxide and sulfone, to leach to groundwater, and the potential for parent terbufos and the sulfoxide and sulfone degradates to runoff to surface water. Terbufos parent degrades rapidly to the sulfoxide and sulfone metabolites, and is considered moderately mobile. Terbufos sulfoxide and sulfone are more mobile and persistent than parent terbufos. The acute DWLOCs calculated for the general U.S. population is 8.1 Fg/L. The chronic DWLOCs calculated for the general U.S. population is 1.7 Fg/L. Maximum acute and	Tefluthrin is immobile in soil and, therefore, will not leach into ground water. Additionally, due to the insolubility and lipophilic nature of tefluthrin, any residues in surface water will rapidly and tightly bind to soil particles and remain with sediment, therefore not contributing to potential Tefluthrin is immobile in soil and, therefore, will not leach into ground water. Additionally, due to the insolubility and lipophilic nature of tefluthrin, any residues in surface water will rapidly and tightly bind to soil particles and remain with sediment, therefore not contributing to potential dietary exposure from drinking water. Plant metabolism studies indicate that tefluthrin per se is not translocated to plants but is degraded in soil to two principal metabolites that are capable of being taken up by plants. EPA has decided that Metabolite VI need not be regulated. Based on tefluthrin not being registered for residentia1 non-food sites, EPA concludes that the aggregate short- and intermediate-term risks do not exceed levels of concern (MOE 1ess than 100), and that there is reasonable certainty that no harm will result from aggregate exposure to tefluthrin residues. *(5*)

		chronic estimated environmental concentrations (EECs) for parent terbufos plus the sulfoxide and sulfone degradates exceed the acute and chronic DWLOCs, respectively, in all cases. *(2)*	
Avian toxicity	Feeding mCry3A plant material to broiler chickens supported growth and mortality rates that were not significantly different than that supported by its isogenic controls (1) Feeding mCry3A grain from event MIR604 to Northern Bobwhite resulted in no adverse effects on mortality, weight gain, and feed consumption. (1) LD_{50} mCry3A > 652 mg protein/ kg body weight	Seven incidents to nontarget terrestrial organisms have been reported. Up to three of the incidents had some indication of misuse or misapplication. All the mortalities involved bird species (mostly raptors), with the exception of one incident involving red wolves in North Carolina, which is believed to be the result of an intentional poisoning. Calculated RQs for birds and mammals significantly exceed EPA's risk concern for both granular formulations. *(2)* Dietary Avian Toxicity: 143 and 157 ppm (from two bobwhite studies). - Avian Reproduction: Terbufos was not considered to produce avian reproductive effects based on results of a bobwhite quail study and a mallard duck study. *(3)*	Low toxicity to birds *(6)*.
Fish toxicity	Feed prepared using plant-produced mCry3A protein to rainbow trout resulted in no adverse effects. (1) Exposure rate = 37.0X EEC	EPA has concerns about risk to nontarget aquatic organisms from parent terbufos and the terbufos sulfoxide and sulfone degradates based on widespread fish kill incidents involving terbufos use on corn with all application methods. These concerns are further supported by standard LOC criteria, which indicate risk concerns to aquatic fish and invertebrates associated with both the clay-based (15%	Highly toxic to fish *(6)*

		active ingredient) and polymer-based (20% active ingredient) granular formulations using banded applications.*(2)* Terbufos ranks fourth in pesticide-induced fish kills reported to the Agency, and is the leading cause of fish kills from use on corn. **Freshwater Fish Acute Toxicity**: Ranges from 0.77 to 20.00 ppb. - Freshwater Invertebrate Acute Toxicity: 0.31 ppb for Daphnia magna. - Marine/Estuarine Fish Acute Toxicity: Data gap.- Marine/Estuarine Invertebrate Toxicity: Data gap. Mollusk toxicity: Data gap *(2)*	
Nontarget and beneficial organisms	mCry3A microbially produced protein were fed to non-target insects and resulted in no adverse effects. (1) **Predatory arthropod, flower bug:** LC_{50} >50 µg mCry3A/g diet; NOEC = 50 µg mCry3A/g diet; Exposure rate ≥ 10.6X EEC (1) **Lady beetle:** LC_{50} >50 µg mCry3A/mL; NOEC = 50 µg mCry3A/mL; Exposure rate ≥ 12.3X EEC (1) **Rove beetle**: **:** LC_{50} >50 µg mCry3A/mL; NOEC = 50 µg mCry3A/mL; Exposure rate ≥	Terrestrial Field Study (Level 1): both soil-incorporated (2 lb ai/A) and nonsoil-incorporated (1 lb/A) resulted in nontarget mortalities, with the latter application much more severe in its effects *(2*,6)	Data not found.

	15.6X EEC (1) **Ground beetle**: **:** LC_{50} >50 µg mCry3A/g blowfly pupa; NOEC = 50 µg mCry3A/g blowfly pupa; Exposure rate ≥ 11.2X EEC (1)		
Honey bee toxicity	**Larval honey bees** were fed microbially-produced mCry3A in a sucrose solution. (1) LC_{50} >50 µg mCry3A/g solutiion; NOEC = 50 µg mCry3A/g solution; Exposure rate ≥ 35.7X EEC (1)	Not described in available studies.	High toxicity to bees *(7)*
Mammalian toxicity	A single dose of mCry3A microbially produced protein was fed to mice and no acute oral toxicity or adverse effects in terms of body weight, detailed clinical observations and gross-pathological lesions were observed. (1) LD_{50} > 2377 mg mCry3A/kg body weight; NOEC = 2377 mg mCry3A/kg body weight;	**Acute Oral**: Toxicity Category I (1.6 and 1.3 mg/kg for male and female rats, respectively). - **Acute Dermal**: Toxicity Category I (0.81 and 0.93 mg/kg for male and female rabbits, respectively). - **Acute Inhalation**: Toxicity Category I (< 0.2 mg/L). - **Delayed Neurotoxicity**: No evidence of acute delayed neurotoxicity at the 40 mg/kg dosage level tested in hens. - **Subchronic Feeding**: The NOEL for both systemic effects and cholinesterase inhibition in a rat subchronic study is 0.25 ppm. - **Subchronic Dermal**: The NOEL for systemic effects in a 30-day rabbit study is 0.020 mg/kg.	**Acute toxicity** studies with the technical grade of the active ingredient tefluthrin: oral LD50 in the rat is 21.8 mg/kg for males and 34.6 mg/kg for females; dermal LD50 in the rat is 316 mg/kg in males and 177 mg/kg in females; acute inhalation LC50 in the rat is 0.037 mg/l and 0.049 mg/l in male and female rats, respectively; primary dermal irritation study in the rabbit showed slight irritation; and the acute delayed neurotoxicity study did not show acute delayed neurotoxicity. In an oral toxicity study, the NOEL for female rats is 100 ppm (equivalent to approximately 5 mg/kg/day). The NOEL for skin effects in rats is 1.0 mg/kg). The NOEL for neurological effects (the observed postural effects) may be between 0.025 and 0.1 mg/kg.

	Exposure rate ≥ 2600X EEC (1)	- **Mutagenicity**: Terbufos did not exhibit mutagenic potential in the Ames assay, the in vivo cytogenetic assay, and the dominant lethal test. - **Teratogenicity**: The NOEL for developmental toxicity in a rat teratology study is 0.1 mg/kg/day. - **Reproduction**: The NOEL for reproductive effects in a three-generation rat reproduction study is 0.25 ppm. - **Oncogenicity**: None *(2*,6)	**Carcinogenicity**: There was no evidence of carcinogenic potential. **Mutagenicity**: There is no mutagenicity concern. **Metabolism**: In both rats and dogs, when given either 1 or 10 mg/kg, most of the radioactivity was found in the feces unchanged and most urinary metabolites were conjugated. In rats, the halflife in the liver is 4.8 days, in the fat is 13.3 days and in the blood is 10.6 days. In a study with rat fat, half of the radioactive residues could be attributed to the parent and the remaining residues consisted of a mixture of fatty acid esters of hydroxylated parent metabolites. **Neurotoxicity**: No acceptable mammalian neurotoxicity studies *(5*). are available.*(5*)
Nontarget soil organism effects	Earthworms were exposed to soil containing microbially produced mCry3A protein and no adverse effects were observed. (1) **Earthworms** LC_{50} >250 µg mCry3A/g moistened soil; NOEC = 250 µg mCry3A/g moistened soil; Exposure rate ≥ 46X EEC (1)	Not described by present reports.	Not found in these reports
Toxicity	Not assigned	Classified by EPA as Toxicity Category I	Toxicity class I for dermal, oral, inhalation exposures, and Class IV for skin irritation.
EDF's Integrated Environmental Rankings - Combined human & ecological scores *(4)*	Not ranked	85-100% where 0 is the lowest and 100 is the highest hazard rating *(4*).	Data lacking; not ranked by any system in Scorecard.

Abbreviations: LD_{50} = Nominal Median Lethal Dose ; LC_{50} = Nominal Median Lethal Concentration; EEC = Estimated Environmental Concentration; NOEC = No Observable Effect Concentration

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