Plant Pest Risk Assessment for MR162 Corn

Syngenta Biotechnology, Inc., has petitioned APHIS (APHIS number 07-253-01p) for a determination that genetically engineered (GE) corn (*Zea mays*) event MIR162 is unlikely to pose a plant pest risk and, therefore, is no longer a regulated article under regulations at 7 CFR part 340. This plant pest risk assessment was conducted to determine whether MIR162 corn is unlikely to pose a plant pest risk. If APHIS determines that MIR162 corn is not a plant pest, APHIS then has no regulatory authority over that organism under its regulations at 7 CFR part 340.

History of Development of MIR162 Lepidopteran-Resistant Corn

Corn is susceptible to attack by a variety of insects from the time it is planted until it is consumed as food or feed (Table 1 on page 12 in petition). Syngenta has developed MIR162 containing an insecticidal protein Vip3Aa20 (Vip = Vegetative insecticidal protein) that is resistant to the feeding damage caused by corn earworm (*Helicoverpa zea*), fall armyworm (*Spodoptera frugiperda*), black cutworm (*Agrotis ipsilon*), and western bean cutworm (*Striacosta albicosta*) larvae. Vip3Aa is produced by the bacterium *Bacillus thuringiensis* (Bt) (Estruch et al. 1996). Bacteria use Vip protein toxins to kill insect prey (Estruch et al. 1996; Schnepf et al. 1998) which then serves as a nutritional source (de Maagd et al. 2001). Vip3Aa proteins are similar to certain Cry proteins (Höfte and Whiteley 1989) and are demonstrated to have toxic effects only on certain insects (Table 2.1 on p. 23 in Carozzi and Koziel 1997).

The mechanism by which Vip proteins exert their insecticidal activity has been studied and found to be similar, but not identical, to that which has been previously described for the Bt Cry proteins that are contained in several commercial insecticide formulations and plants engineered for insect resistance. The Vip and Cry proteins bind to different receptors in the insect (Lee et al. 2003), and the insecticidal activity of Vip3Aa proteins is limited to species within selected families of the order Lepidoptera (Table 27 on pages 74-75 in petition). For example, the Vip3Aa protein in MIR162 does not provide corn plants protection against damage caused by European corn borer (*Ostrinia nubilalis*) and corn root worm (*Diabrotica sp*), the most widespread and damaging insect pests of maize in the U.S. Corn Belt. According to Syngenta, when MIR162 corn hybrids containing Vip3Aa are combined with European corn borer protected corn, such hybrids have the potential to provide growers the means of protecting their corn crops from damage caused by a broader range of lepidopteran pests (pages 74-75 in petition).

MIR 162 corn has been field tested under APHIS regulations since 1999. Data were provided in the petition for field trials completed prior to the petition submission.

Tolerance exemptions and conditional pesticide registrations have been granted for the plant-incorporated protectant in MIR162 corn and the genetic material necessary for its production. On August 6, 2008, the Environmental Protection Agency (EPA) granted an exemption from the requirement of a tolerance for residues of Vip3Aa proteins (including

the Vip3Aa20 variant) in or on food and feed commodities of corn (73 FR 45620-45624). Likewise, on April 30, 2009, EPA also approved the conditional registrations of Vip3Aa20 produced in MIR 162 corn for use as lepidopteran insecticide (74 FR 19956-19957). An exemption from the requirement of tolerance has been established for the selectable marker gene phosphomannose isomerase (PMI) protein in all crops (69 FR 26770-26775). Syngenta's food safety summary submitted to FDA indicated that food and feed derived from corn event MIR162 are as safe and nutritious as food and feed derived from conventional corn. At the conclusion of their consultation with FDA on December 9, 2008, the FDA concluded that it had "no further questions concerning grain and forage derived from corn event MIR162" (FDA BNF No. 000113).

Description of Inserted Genetic Material

MIR162 maize was produced by transformation of immature maize embryos using an *Agrobacterium tumefaciens* plant-pathogenic bacterium vector system that is disarmed of DNA sequences within the T-DNA (transfer-DNA), which upon integration into the plant genome of infected cells are normally responsible for the formation of crown gall tumors in plants. The disarmed *Agrobacterium tumefaciens* harbors a plasmid vector pNOV1300 that contained within its T-DNA *vip3Aa19* and *manA* gene expression cassettes.

The first expression cassette consists of four genetic elements:

- *Z. mays* polyubiquitin promoter and first intron (ZmUbiInt). This promoter provides constitutive expression in monocots (Christensen et al. 1992).

- Full length *Vip3Aa19* gene. This protein coding region is a variant of the native *vip3Aa1* gene (Estruch et al. 1996) from *B. thuringiensis* strain AB88. The *vip3Aa19* gene was codon optimized for expression in maize (Murray et al. 1989). The *vip3A19* encodes a Vip3Aa19 protein that has insecticidal activity against many lepidopteran insect pests.

- Intron #9 from the phosphoenolpyruvate carboxylase gene (iPEPC9) from *Z. mays* (Hudspeth and Grula 1989).

- 35S RNA Terminator sequence from the cauliflower mosaic virus genome. This sequence contains signals for termination of transcription and directs polyadenylation (Franck et al. 1980).

The second expression cassette consists of three genetic elements:

- ZmUbiInt (same as above).

- A *manA* gene from *E. coli* strain K-12. This gene encodes the enzyme phosphomannose isomerase (PMI) that catalyzes the interconversion of mannose-

6-phosphate to fructose-6-phosphate (Negrotto et al. 2000), which was used as a selectable marker during transformant selection.

- NOS (Nopaline Synthase) gene terminator sequence from *A. tumefaciens.* This terminates gene expression by a polyadenylation site (Depicker et al. 1982).

The production of PMI enzyme in the transformed tissue allows corn tissue containing the *vip3Aa19* and *manA* gene expression cassettes to be selected on medium containing the sugar mannose. The *manA* gene expression, which produces phosphoisomerase enzyme, confers no other benefit to the regenerated transformed corn plant. Syngenta provided evidence demonstrating that the final product does not contain any of the backbone sequences outside of the T-DNA borders from the transformation vector, pNOV1300.

Southern blot analyses and nucleotide sequencing demonstrated that MIR162 corn contains a single intact T-DNA insert in the corn genome. Furthermore, Southern blot analyses also demonstrated that the T-DNA insert contains: i) single copies of a *vip3Aa* gene and a *manA* gene; ii) two copies of the ZmUbiInt promoter; iii) one copy of the NOS terminator; and iv) no backbone sequences from transformation plasmid pNOV1300. Nucleotide sequencing additionally determined that the MIR162 maize T-DNA insert did not locate within any known *Z. mays* gene. Further, no novel open reading frames were created that spanned either the 5′ or 3′ junctions between the T-DNA and *Z. mays* genomic sequences.

Syngenta's characterization of the T-DNA in MIR 162 and Mendelian inheritance of transgene segregation data provide evidence for the functional stability and intactness of the two transgene coding sequences over several breeding generations during the development of MIR162 corn hybrids (pages 23- 46 in petition). However, the *vip3A19* coding sequence analysis also revealed two single nucleotide changes in the coding sequence contained in the MIR162 maize T-DNA, as compared with the sequence present in the transformation plasmid pNOV1300. The new gene variant incorporated into the MIR162 maize genome has been designated as *vip3Aa20*. One of the mutations resulted in a single codon change for the amino acid originally encoded, while the other mutation was a silent mutation (i.e., the amino acid produced did not change). Mutational changes in genetic elements are very common and are the ultimate source of genetic variation found in nature, and the single functional mutational change in the *vip3Aa19* gene that resulted in *vip3Aa20* is within the range of mutation rates observed in nature for plants (Kovalchuk et al. 2000). The single amino acid substitution between *vip3Aa19* and *vip3Aa20* occurs at position 129 (see the petition pages 47-48 for a detailed description). Based on Syngenta's laboratory bioassay results, the amino acid differences between *vip3Aa19* and *vip3Aa20* variants do not impact insecticidal activity against target insect pests, as the position 129 occurs outside of the core protein domain involved in insecticidal activity (Estruch and Yu 2001; Lee et al. 2003). These data also indicate that no novel proteins, other than Vip3Aa20 and PMI, will be produced in MIR162 maize. These genetic characterization data demonstrate that, apart from the well-characterized change that resulted in a single altered amino acid in the *vip3Aa19* coding

sequence, there are no unintended changes in the MIR162 corn genome as a result of the T-DNA insertion (see pages 23-46 of petition).

Plant Pest Risk Assessment

MIR162 maize was produced by transformation of corn tissue using *A. tumefaciens* to introduce a gene that confers tolerance to certain lepidopteran (caterpillar) pests of corn. Because *A. tumefaciens* is a plant pest and some of the regulatory sequences used to facilitate expression of these genes in corn were derived from plant pests, the engineered corn has been considered a regulated article under APHIS regulations at 7 CFR part 340. APHIS administers these regulations under the authority of the Plant Protection Act of 2000 (PPA) (7 U.S.C. Sec 7701 *et seq*.). APHIS' authority to regulate genetically engineered organisms under the PPA is limited to those GE organisms that are plant pests as defined under Section 14 of the PPA. APHIS regulations under 7 CFR part 340.1 defines a plant pest 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."

Potential impacts to be addressed in this risk assessment are those that pertain to the use of MIR162 corn and its progeny in the absence of confinement. Of the information requested by APHIS for submission of a petition for nonregulated status (§ 340.6(c)(4)), APHIS examined information submitted by the applicant related to plant pest risk characteristics, disease and pest susceptibilities, expression of the gene product, new enzymes or changes to plant metabolism, weediness of the regulated article, and any impacts on the weediness of any other plant with which it can interbreed. Furthermore, APHIS examined the effects of the regulated article on nontarget organisms, as MIR162 corn is genetically engineered to produce a lepidopteran-specific toxin. Issues related to agricultural or cultivation practices are in the Environmental Assessment for MIR162 corn.

Potential Impacts of Genetic Modifications on Altered Disease and Pest Susceptibilities

USDA-APHIS assessed whether MIR162 corn is likely to have significantly increased disease and pest susceptibility. This assessment encompasses a thorough consideration of introduced traits and interactions with pests and disease.

Corn (*Zea mays* ssp. *mays*) is not a plant pest in the United States (USDA-APHIS 2000). Furthermore, none of the sequences derived from the plant pests (*Agrobacterium* and CaMV) that were incorporated into MIR 162 corn result in the production of infectious agents or disease symptoms in plants, and so they are unlikely to pose a plant pest risk. The description of the genetic modifications, including genetic elements, expression of the gene product and their functions for MIR162 corn has been summarized above.

Syngenta routinely monitors their corn field trials for the fungal diseases gray leaf spot, northern corn leaf blight, and southern corn leaf blight. The corn insects monitored include corn rootworm, corn flea beetle, grasshopper, stink bug, and other coleopteran beetles (personal communication to Subray Hegde, APHIS, BRS 8/27/09). The data submitted by Syngenta indicated no meaningful differences between MIR162 corn and the non-transgenic counterparts for disease, such as grey leaf spot disease (petition Tables 22 and 23, pp. 65-66, and Supplement to Petition, Tables S-5 and S-6, pp. 7-8), and non-targeted insect pests (field test reports submitted to APHIS/BRS on notifications and permits, Table A.1, pages 127-128 in petition).

The data presented in the petition indicate no difference in compositional and nutritional quality of MIR162 corn compared to conventional corn, apart from the presence of Vip3Aa20 and PMI proteins. Although some of the variables measured by the applicant showed statistically significant differences between MIR162 corn and the non-transgenic hybrid controls (Table 26 on page 72 in petition), none of the values for the forage and grain composition characteristics were outside the range of natural variability of conventional corn reported by the International Life Sciences Institute Crop Composition Database (Ridley et al. 2004; ILSI 2006) or in the OECD consensus document on corn composition (OECD 2003). Therefore, the composition of MIR162 corn is not biologically different than conventional corn (with the exception of the Vip3Aa20 and PMI proteins). Based on the known functions and mechanisms of actions of these proteins (summarized in the petition), neither of these proteins are expected to directly alter susceptibility to plant pathogens. Thus MIR 162 corn is expected to be susceptible to the same plant pathogens as conventional corn.

The Vip3Aa20 protein will decrease the susceptibility of MIR 162 corn to certain targeted insect pests (as noted below under *Potential Impacts on Target and Nontarget Organisms*), which could indirectly affect populations of other insect pests on corn, and likewise plant pathogens that infect corn as a result of feeding damage. As noted in the petition (pg. 13) insects pests of corn play an important role in the transmission and dissemination of pathogenic organisms during maize development. According to the Petitioner, "Ear, kernel, and cob rots occur wherever maize is grown and can result in reduced test weight, poor grain quality, and mycotoxin contamination of food and feed. *Fusarium* kernel or ear rot is the most widespread disease of maize ears and is frequently associated with insect feeding damage." They indicate that although crop losses attributable to *O. nubilalis* and *Diabrotica* infestations have been well characterized and are significant, there is not as much quantitative information available on the economic impacts of other major insect pests of maize, specifically the leaf and ear-feeding insects *H. zea*, *S. frugiperda*, *A. ipsilon*, and *S. albicosta,* which are the primary target pests of Vip3A120 in MIR 162 corn. These pests are not as widespread as some corn pests, but crop infestations by these pests have the potential to significantly lower grain yield and quality. Data in the petition (petition Table 22 and 23, pp. 65-66) showed no significant difference in grain yield between a MIR 162 corn hybrid and a near isogenic control line in two years of field trials in a variety of US maize growing locations (6-10 locations per year) (petition Table 21, pg. 63). These data indirectly support that MIR 162 corn does not have increased susceptibility to insects or pathogens that directly or indirectly affect

yield. In a variety of field studies, other insect protected corn expressing Bt proteins have been shown to have significantly lower levels of common mycotoxin that are produced by fungal pathogens (Wu 2006); however, no data were provided on mycotoxin levels in MIR 162 corn.

Potential Impacts from Outcrossing (Gene Flow) to Sexually-compatible Wild Relatives

Gene flow is a natural biological process with significant evolutionary importance. A number of angiosperm taxa are believed to be derived from hybridization or introgression between closely related taxa (Grant 1981; Soltis and Soltis 1993; Rieseberg 1997; Hegde et al. 2006), and even in the existing floras, the occurrence of hybridization or introgression is reported to be widespread (Knobloch 1972; Stace 1987; Rieseberg and Wendel 1993; Peterson et al. 2002). It has been a common practice by plant breeders to artificially introgress traits from wild relatives into crop plants to develop new cultivars. However, gene flow from crops to wild relatives is also thought of as having a potential to enhance the weediness of wild relatives, as observed in rice, sorghum, sunflower and few other crops (see Table 1 in Ellstrand et al. 1999).

APHIS evaluated the potential for gene introgression to occur from MIR162 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* (OECD 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. In the U.S. a fairly rare, sparsely dispersed feral population of teosinte has been reported in Florida (USDA Plant database: http://plants.usda.gov/java/county?state_name=Florida&statefips=12&symbol=ZEME).

The genus *Tripsacum* contains up to 16 recognized species, most of which are native to Mexico, Central and South America, but three (*T. dactyloides, T. floridatum*, and *T. lanceolatum*) exist as wild and/or cultivated species in the continental U.S (OECD 2003); and two taxa (*T. fasciculatum* and *T. latifolium*) also occur in Puerto Rico (PLANTS Database, accessed 7/13/2009). Though many of these species occur where corn might be cultivated, gene introgression from MIR162 corn under natural conditions is highly unlikely. 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 of them can 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, even in those instances of accidental gene flow between MIR162 corn and wild relatives, the transgenes of MIR162 corn are unlikely to transform corn wild relatives into more weedy species.

Introgression of genes from corn into teosinte or *Tripsacum* species has not been described to occur in nature in the U.S. While some teosinte may be considered weeds in certain instances, they are also used by some farmers for breeding improved maize (Sánchez Gonzàlez and Ruiz Corral 1997 and references therein). Teosinte is described as being susceptible to many of the same pests and diseases that attack cultivated corn (Sánchez Gonzàlez and Ruiz Corral 1997). In the wild, introgressive hybridization from corn to teosinte is currently limited, in part, by several factors including geographic isolation, differing degrees of genetic incompatibility, differences in flowering time in some cases, developmental morphology and timing of the reproductive structures, dissemination, and dormancy (Doebley 1990a and 1990b; Galinat 1988; Ellstrand 2007). First-generation hybrids are generally less fit for survival and dissemination in the wild, and show substantially reduced reproductive capacity, which thus acts as a significant constraint to introgression. Data included in the petition demonstrated that there were no significant differences in viability and morphology of pollen from collected from greenhouse-grown MIR162 hybrid plants and near-isogenic control plants (petition, Table 24 and Figure 20, pp. 67-68.); therefore, the outcrossing rate of MIR 162 corn is not expected to be any different from other corn. Based on the data presented in the petition, MIR162 corn does not exhibit characteristics that cause it to be any weedier than other cultivated corn (see below). Moreover, its potential impact due to the extremely limited potential for gene introgression into teosinte is not expected to be any different than that of other cultivated corn varieties.

Based on the above considerations, MIR162 corn will not adversely impact sexually compatible wild relatives or their weediness characters.

Potential Impacts Based on the Relative Weediness of MIR162 Corn

In the U.S., corn is not listed as a weed (Crockett 1977; Holm et al. 1979; Muenscher 1980), nor is it present on the Federal noxious weed list (7 CFR part 360; http://www.aphis.usda.gov/plant_health/plant_pest_info/weeds/downloads/weedlist2006.pdf). Furthermore, corn is grown throughout the world without any report that it is a serious weed or that it forms persistent feral populations. Like many domesticated crops, corn seed from a previous year's crop can overwinter and germinate the following year. For instance, the appearance of corn seedlings in soybean fields following a corn crop is a common occurrence. Manual or chemical measures are often applied to remove these volunteers, but the plants that are not removed do not typically result in feral populations in following years. Corn also possesses few of the characteristics of plants that are notably successful weeds (Baker 1965; Keeler 1989).

APHIS assessed whether MIR162 corn is any more likely to become a weed than the isogenic nontransgenic corn line, or other corn varieties currently under cultivation. The assessment encompasses a thorough consideration of the basic biology of corn and an evaluation of the unique characteristics of MIR162 corn evaluated under field conditions. Syngenta conducted agronomic field trials during the 2005 and 2006 growing seasons across 6-10 locations representative of the major corn-growing areas of the upper mid-west U.S. For the majority of the traits assessed, there were no statistically significant

differences between MIR162-derived hybrids and their control counterparts. Results of the 2005 agronomic equivalence trials presented in Table 22 of the petition revealed only one statistically significant difference between a control and MIR162 variant - the average number of germinated plants per plot was slightly higher in the MIR162 plots compared to control plots. But the difference was only 3.2%, and is not considered to be of biological significance because the effect was not repeated in the 2006 trials, and there was no effect of genotype observed in the seed germination and dormancy study (see Tables 18 and 19). No other differences in phenotypic characteristics that might contribute to enhanced weediness were observed between MIR162 and control lines for the wide range of phenotypic endpoints assessed in these trials or in greenhouse or laboratory experiments (see petition Table 16, pp. 57-58, for the full list of characteristics evaluated). These characteristics covered seed germination and dormancy, emergence, vegetative and reproductive growth, seed retention, and plant-ecological interactions. Furthermore, the genes inserted into MIR 162 corn do not confer tolerance to herbicides; therefore, there is no change in the ability to control MIR 162 corn as a weed on agricultural land as a result of the insertion of the foreign genes.

Based on the agronomic field data and literature survey about corn weediness potential, MIR162 corn lacks ability to persist as troublesome weed, and there would be no direct impact on current weed management practices for corn cultivation.

Potential Impacts on Target and Nontarget Organisms, Including Beneficial Organisms

The mechanism by which Vip proteins exert their insecticidal activity has been studied and found to be similar, but not identical, to that which has been previously described for the Cry proteins. For many decades microbial products containing Bt (the organism that produces the Cry1A protein) have been used to control insect pests on a commercial scale and for home garden applications (Glare 2000; Shelton 2002). Plants that were genetically engineered to express the Cry1A protein have a history of safe use in the U.S. Since the mid-1990s, corn and cotton lines have been commercialized without substantiated reports of significant deleterious impacts on non-target organisms (EPA 2008; OECD 2007).

Vip3Aa has activity against several of the major lepidopteran pests of corn, specifically: *A. ipsilon*, *H. zea*, *S. albicosta*, and *S. frugiperda* (Table 27 on pages 74-75 in petition). Syngenta summarized data from field efficacy trials comparing the activity of MIR162 with that of Bt11 corn, hybrids of Bt 11 x MIR162, and conventional insecticide (*Warrior® Insecticide*) treated corn, relative to untreated controls, with regard to feeding damage from these insects (Petition Figure 21 on page 76 in petition). MIR162 alone has no activity against *O. nubilalis* but is efficacious in limiting feeding damage caused by the other four insect pests (mean damage ratings were at most 30% of that of the untreated controls in Petition Figure 21 on page 76 in petition). Whereas Bt11 corn lines (with Cry proteins) are highly efficacious against *O. nubilalis*, they have limited or no activity against the other four insects. According to Syngenta, the combined-trait

Bt11xMIR162 hybrids are very efficacious against all five insects (mean damage ratings were at most about 10% of untreated controls (Figure 21 on pg. 76 in petition).

The Bt Toxin Nomenclature Committee currently lists 25 variants of the Vip3Aa protein. This narrow spectrum of activity for Vip3Aa proteins is a positive attribute from an ecological perspective, as maize hybrids containing a Vip3Aa protein are unlikely to pose a risk to nontarget organisms inhabiting maize ecosystems. Like Cry proteins, Vip3Aa proteins are not expected to adversely affect non-target invertebrates, such as bees, and vertebrate organisms, including birds, mammals and humans, because they do not contain the receptor found in the midgut of target insects. Data provided in the petition (summarized in the Table 31, pg. 89) confirmed that in the bird, mammal, honey bee, above ground arthropod, and soil dwelling invertebrate studies, no observable adverse effects or differences in survival were noted at doses of Vip3A proteins that were well above those expected from exposure to the Vip3Aa20 protein from MIR162 planted in the field. The nontarget above-ground arthropods and soil-dwelling invertebrates studied (lady bird beetles, green lacewings, minute pirate bugs, collembola, earthworms, and rove beetles) were considered to be representative of the corn agro-ecosystem.

Although not an endangered or threatened species, *Danaus plexippus* (monarch butterfly) is a species of high conservation interest, and there has been concern that it may be harmed by consuming pollen from transgenic insect-protected maize. The monarch is susceptible to Cry1Ab (Hellmich et al. 2001), the most common insecticidal protein in transgenic maize. However, the distribution of the monarch's food plant (*Asclepias syriaca* - common milkweed), its pattern of migration, and the timing of maize anthesis means that very few monarchs are exposed to harmful concentrations of Cry1Ab (Sears et al. 2001).

The exposure assessments used to assess the risks of maize containing Cry1Ab to monarchs are also valid for MIR162 maize. In addition, it has been shown that monarchs are not susceptible to Vip3Aa1. Lee et al. (2003) showed that trypsin protease digested Vip3Aa1 did not form pores in the midgut of monarchs; pore formation appears to be essential for toxicity and occurs in the guts of insects susceptible to Vip3Aa1. These investigators also found no mortality of monarch butterfly in a surface diet bioassay limit test at 1000 ng/cm^2. MIR162 corn, therefore, poses low risk to monarchs because of minimal hazard of Vip3Aa20 and low exposure to Vip3Aa20-containing pollen. Besides, the restriction of toxicity of Vip3Aa20 to Lepidoptera, and the minimal exposure of endangered Lepidoptera to maize, indicates that Vip3Aa20 in MIR162 maize is expected to have no harmful effects on any endangered or threatened species in the U.S.

Potential Impacts from Transferring Genetic Information from MIR162 Corn to Organisms with which It cannot Interbreed

APHIS examined the potential for the new genetic material inserted into MIR162 corn to be horizontally transferred to other organisms without sexual reproduction and whether such an event could lead directly or indirectly to disease, damage, injury or harm to plants, including the creation of more virulent pathogens. The horizontal gene transfer between unrelated

organisms is one of the most intensively studied fields in the bio-sciences since 1940, and the issue gained extra attention with the release of transgenic plants into the environment (Droge et al. 1998). Potential risks from stable horizontal gene transfer (HGT) from genetically engineered organisms to another organism without reproduction or human intervention was recently reviewed (Keese 2008). Mechanisms of HGT include conjugation, transformation and transduction, and other diverse mechanisms of DNA and RNA uptake and recombination and rearrangement, most notably through viruses and mobile genetic elements. HGT has been a major contributor to the spread of antibiotic resistance amongst pathogenic bacteria and the emergence of increased virulence in bacteria, eukaryotes and viruses and in the long run has contributed to major transitions in evolution.

Potential for Horizontal Gene Transfer to Bacteria or Fungi

The MIR 162 has two bacteria genes. Horizontal gene transfer and expression of DNA from a plant species to other bacterial species is unlikely to occur based on the following observations. Although there are many opportunities for plants to directly interact with fungi and bacteria (e.g. as commensals, symbionts, parasites, pathogens, decomposers, or in the guts of herbivores), there are almost no evolutionary examples of HGT to bacteria from eukaryotes or from plants to fungi (as reviewed in Keese 2008). The only genes likely to be transferred successfully from genetically engineered plants to bacteria are other bacterial genes. Horizontal transfer from and expression in bacteria of the foreign DNA inserted into the nuclear genome of MIR162 corn is unlikely to occur. First, many genomes (or parts thereof) have been sequenced from bacteria that are closely associated with plants including *Agrobacterium* and *Rhizobium* (Kaneko et al. 2000; Wood et al. 2001; Kaneko et al. 2002). There is no evidence that these organisms contain genes derived from plants. Second, in cases where review of sequence data implied that horizontal gene transfer occurred, these events are inferred to occur on an evolutionary time scale on the order of millions of years (Koonin et al. 2001; Brown 2003). Third, transgene DNA promoters and coding sequences are optimized for plant expression, not prokaryotic bacterial expression. Thus even if horizontal gene transfer occurred, proteins corresponding to the transgenes are not likely to be produced. Fourth, the FDA has evaluated horizontal gene transfer from the use of antibiotic resistance marker genes and concluded that the likelihood of transfer of antibiotic resistance genes from plant genomes to microorganisms in the gastrointestinal tract of humans or animals, or in the environment, is remote (http://vm.cfsan.fda.gov/~dms/opa-armg.html).

Potential for Horizontal Gene Transfer to Viruses

APHIS also considered whether horizontal transfer of DNA from MIR162 corn to plant viruses was likely to occur and would lead to the creation or selection of a more virulent plant pathogen through recombination with other plant viruses. This issue has been considered before by other science review panels and government regulatory bodies (for a general review of the issue see Keese 2008). The only virus sequence contained within MIR 162 corn is the 35S RNA terminator and this has not been implicated in viral recombination.

Therefore, APHIS concludes that horizontal gene transfer is unlikely to occur and thus poses no significant environmental or plant pest risk. Finally, under natural conditions; no

transfer of an intact functional gene has been demonstrated to date (Miki and McHugh 2004). Therefore APHIS concludes that horizontal gene transfer is unlikely to occur and thus poses no significant environmental or plant pest risk.

Conclusion

APHIS has reviewed and conducted a plant pest risk assessment on MIR162 corn. Due to the lack of plant pest risk from the inserted genetic material, the lack of weediness characteristics of MIR162 corn, the lack of atypical responses to disease or plant pests in the field, the lack of deleterious effects on non-targets or beneficial organisms in the agro-ecosystem, and the lack of horizontal gene transfer, APHIS concludes that MIR162 corn is unlikely to pose a plant pest risk.

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