

Plant Pest Risk Assessment for Pioneer 4114 Maize

Background

Pioneer Hi-Bred International, Inc. (referred to as “Pioneer” hereafter) has petitioned APHIS (USDA-APHIS Petition Number #11-244-01p, referred to as “Petition”) for a determination that genetically engineered (GE) corn (*Zea mays*) event DP-ØØ4114-3 (referred to as “4114 maize” or “4114”) is unlikely to pose a plant pest risk and, therefore, is no longer a regulated article under regulations at 7 CFR part 340. APHIS administers 7 CFR part 340 under the authority of the plant pest provisions of the Plant Protection Act of 2001¹. This plant pest risk assessment was conducted to determine whether event DP-ØØ4114-3 maize is unlikely to pose a plant pest risk.

History of Development of Insect-Resistant and Herbicide-Tolerant 4114 maize

The United States is the largest producer of corn in the world (USDA-ERS, 2010). Corn is the primary feed grain in the United States, accounting for more than 90 percent of the total value of feed grains (USDA-ERS 2010). Corn is also considered as a major biofuel crop in the U.S. Recently strong demand for ethanol production has resulted in increased corn demand and higher corn prices and has provided incentives to increase corn acreage. According to USDA-NASS (<http://www.usda.gov/nass/PUBS/TODAYRPT/acrg0612.pdf>) “Corn production in the U.S. has risen over time and is currently about 96 million acres in 2012”. USDA has projected that U.S. corn cultivation may continue to increase in the coming years (USDA-NASS 2013).

Maize producers face substantial economic losses from insect damage every year. For centuries, scientists have searched for crop plants that can survive and produce in spite of insect pests. Mycogen Seeds introduced the first genetically engineered corn to express the insecticidal protein that occurs naturally in the soil bacterium *Bacillus thuringiensis* (Bt) in 1996. Now, Bt corn is one of the most widely adopted genetically engineered crops. In 2009, 63% of field corn planted in the United States was transgenic for Bt traits.

Bt is a naturally-occurring soil borne bacterium that is found worldwide. This bacterium produces crystal-like proteins (“Cry” proteins) that selectively kill specific groups of insects. Once eaten by the insects, Cry proteins bind to specific “receptors” on the intestinal lining and rupture the cells. If enough toxins are eaten, the specific insects will die. Bt corn was created by inserting selected cry genes into the corn plant.

The 4114 maize is an insect-resistant and herbicide tolerant product. Pioneer used recombinant DNA techniques to develop 4114 maize event which contains *cry1F*, *cry34Ab1*, *cry 35Ab1* and *pat* genes. The *cry1F* gene derived from the bacterium *Bacillus thuringiensis* subsp. *aizawai*. This gene encodes a Cry1F insecticidal protein that controls specific lepidopteran pests of corn.

¹ Section 403 (14) of the Plant Protection Act (7USC Sec 7702(14) defines plant pest as: “Plant Pest - The term “plant pest” means any living stage of any of the following that can directly or indirectly injure, cause damage to, or cause disease in any plant or plant product: (A) A protozoan. (B) A nonhuman animal. (C) A parasitic plant. (D) A bacterium. (E) A fungus. (F) A virus or viroid. (G) An infectious agent or other pathogen. (H) Any article similar to or allied with any of the articles specified in the preceding subparagraphs.”

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The Cry1F and associated genetic elements from 4114 maize are identical to those in 1507 maize, which was given non-regulated status by USDA-APHIS in 2001 (Petition 00-136-01p). Both *cry34Ab1* and *cry35Ab1* genes are derived from the bacterium *Bacillus thuringiensis* strain PS149B1. The Cry34Ab1 and Cry35Ab1 proteins together comprise an active binary insecticidal crystal protein that confers resistance to certain corn rootworm pests. The *cry34Ab1* and *cry35Ab1* and associated genetic elements from 4114 are identical to those in 59122 maize, which was deregulated by USDA-APHIS in 2005 (Petition 03-353-01p). The 4114 also expresses the *pat* gene, which is derived from the bacterium *Streptomyces viridochromogenes*. This gene encodes a phosphinothricin-N-acetyltransferase (PAT) enzyme. PAT detoxifies glufosinate and thereby confers tolerance to herbicides based on this active ingredient. The herbicide tolerance provides a weed management tool for farmers and a method of selecting for transgenic corn in the laboratory.

The 4114 maize is a new maize line that combines genes and traits from two maize lines that had previously been given non-regulated status: 1507 maize deregulated by USDA in 2001, which expresses the Cry 1F and PAT proteins, and 59122 maize deregulated by USDA in 2005, which expresses the Cry 34Ab1, Cry 35Ab1 and PAT proteins. From the breeding stack of these two lines (1507 maize and 59122 maize), 1507 x 59122 maize was developed and is grown widely in the U.S. Globally, many countries and the European Union have approved and used 1507 maize as food/feed since 2002 and have approved and used 59122 since 2005 (ISAAA Briefs 39 2008).

4114 and 1507 x 59122 have identical genes and their genetic elements, but the *cry* and *pat* genes for 1507 x 59122 maize are located in two unlinked loci. The purpose in developing 4114 is to have all these inserted genes on a single transformation construct that has been integrated at a single genetic locus in the maize genome. 4114 will have an advantage over 1507 x 59122 maize because having three linked traits at a single locus will simplify breeding efforts.

APHIS BRS completed plant pest risk assessments and Environmental Assessments (EAs) for 1507 maize and 59122 maize (http://www.aphis.usda.gov/biotechnology/not_reg.html). The EAs fully addressed all resource areas of potential concern. In both petitions, APHIS concluded on the basis of the EA that the impacts would not be significant. The agency issued Findings of No Significant Impacts (FONSI) and made determinations of nonregulated status for each. The Cry1F, Cry34Ab1, Cry35Ab1 and PAT proteins have a history of safe use in agricultural crop commodities. Cry1F protein and PAT protein have been present in commercial maize varieties such as 1507 since 2003. Cry34Ab1, Cry35Ab1 and PAT protein have been present in commercial maize varieties such as 59122 and 1507 x 59122 maize since 2006. PAT protein has been present in a numerous commercial GE crops planted in the U.S since 1996.

Description of the Modification—Genetic material inserted and protein produced

4114 maize was produced using *Agrobacterium tumefaciens* mediated transformation of Pioneer proprietary inbred line PHWWE (Pioneer 2011, pp. 30-34). The *A. tumefaciens* strain LBA4404 that was used to develop 4114 maize was made nonpathogenic by removing tumor inducing (Ti) DNA sequences normally present in *A. tumefaciens* (Koncz and Schell 1986). The

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disarmed *A. tumefaciens* carried a binary plasmid vector PHP27118 (Pioneer 2011, Figure 3 and 4, pp. 30-31). In vitro selection of transformation events was based on tolerance to the herbicide bialaphos which is rendered non-toxic to plant tissues by the presence of the PAT protein. The size of the T-DNA was 11,978 base pairs (bp) and contained a single T-DNA delineated by left and right border regions in which there were four expression cassettes: *cryIF* gene cassette, *cry34Ab1* gene cassette, *cry35Ab1* gene cassette and the *pat* gene cassette.

The *cryIF* expression cassette consisted of the following genetic elements (Pioneer 2011, Table 2, p.32):

- Promoter, 5' untranslated region (UTR) and intron from the maize polyubiquitin gene (Christensen et al., 1992).
- Truncated version of the *cryIF* gene from *Bacillus thuringiensis* var. *aizawai*
- Terminator sequence from the *Agrobacterium tumefaciens* pTi15955 ORF 25 (Barker et al., 1983).

The *cry34Ab1* expression cassette consisted of the following genetic elements (Pioneer 2011, Table 2, page 33):

- Promoter, 5' untranslated region (UTR) and intron from the maize polyubiquitin gene (Christensen et al., 1992).
- Codon-optimized version of the *cry34Ab1* gene encoding 14KDa Delta-endotoxin parasporal crystal protein from *Bacillus thuringiensis* strain PS149B1 (Ellis et al., 2002).
- Terminator region from *Solanum tuberosum* proteinase inhibitor II gene (*pinII*) (Keil et al., 1986).

The *cry 35Ab1* expression cassette consisted of the following genetic elements (Pioneer 2011, Table 2, page 34):

- Promoter from *Triticum aestivum* peroxidase including leader sequences (Hertig et al., 1991).
- Codon-optimized version of the *cry35Ab1* gene encoding a 44kDa delta-endotoxin parasporal crystal protein from *Bacillus thuringiensis* strain PS149B1 (Ellis et al., 2002).
- Terminator region from *Solanum tuberosum* proteinase inhibitor II gene (Keil et al., 1986).

The *pat* gene expression cassette consisted of the following genetic elements (Pioneer 2011, Table 2, page 34):

- Promoter (CaMV 35S) from Cauliflower Mosaic Virus (Franck et al., 1980).
- Codon-optimized phosphinothricin acetyltransferase (*pat*) gene from *Streptomyces viridochromogenes*.
- 35S terminator from Cauliflower Mosaic Virus (Franck et al., 1980)

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In addition to the above-mentioned genetic elements, the inserted T-DNA also contains short noncoding DNA sequences called polylinkers. The polylinkers contain restriction enzyme recognition sites and are used for cloning purposes. Also, the T-DNA left and right border sequences (T-DNA borders) contain 25 base pairs of DNA that are leftover from the Ti plasmid of *A. tumefaciens*.

Data from Southern blot analyses provided to and reviewed by APHIS, demonstrated that a single, intact PHP27118 T-DNA (Pioneer 2011, Figure 13, page 47) was inserted into the genome of 4114 maize and that no region from the backbone of plasmid PHP27118 was inserted (Pioneer 2011, page 43-74). The stability of the introduced genes was determined by event-specific and gene-specific endpoint PCR analyses for several generations ((Pioneer 2011, pp. 75-76). The stability integration was further confirmed by the Mendelian inheritance of the T-DNA in 4114 maize over four generations (Pioneer 2011, pp. 75-76).

Potential for 4114 Maize to have Altered Disease and Pest Susceptibilities

USDA-APHIS assessed whether 4114 maize is likely to have significantly increased disease and pest susceptibility. This assessment encompasses a thorough consideration of the transformation process, introduced genes and their genetic elements, and their expression products to cause interactions with pests and diseases.

Transformation Process

APHIS considered the potential for the transformation process to cause or aggravate disease symptoms in 4114 maize or other plants or to cause the production of plant pathogens. Wild type *Agrobacterium tumefaciens* carries a tumor-inducing (Ti) plasmid that can be transferred to broadleaf plants and cause crown gall disease. *Agrobacterium tumefaciens* strain LBA4404 contains a disarmed Ti plasmid that is incapable of inducing tumor formation due to the deletion of the phytohormone genes originally present in the *Agrobacterium* plasmid (Koncz and Schell 1986). The 4114 maize was produced by an *Agrobacterium tumefaciens* strain LBA4404 mediated transformation with plasmid PHP27118 (Pioneer 2011, Page 38). This transformation process should not lead to crown gall disease in 4114 maize. Instead, in the T-DNA region maize-optimized *cry1F*, *cry34Ab1* and *cry35Ab1* genes, as well as the synthetic *pat* gene and their regulatory components necessary for their expression in the maize genome were introduced into maize. *Agrobacterium*-mediated plant transformation has been used widely for decades, has not been implicated in causing plant disease, and is highly unlikely to pose a plant pest risk.

Introduced genes and their genetic elements

APHIS reviewed Southern blot analysis data that demonstrates that 4114 maize plants regenerated from the transformation event contain single copy, intact plasmid PHP27118 T-DNA. No region from the backbone of plasmid PHP27118 was inserted (Pioneer 2011, pp. 43-73). The stability of the inserted DNA was also evaluated over several generations (Pioneer 2011, pp. 75-76). Plant phenotypes were evaluated to ensure stability of the traits during the plant breeding process. Mendelian segregation of the inserted genes was analyzed using Chi-square analysis over 5 generations (Pioneer 2011, p. 76). Each generation of plants was treated

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with glufosinate-ammonium to eliminate those plants that were not herbicide-tolerant, reflecting a lack of inheritance of the *pat* gene. Table 7 of the Petition shows the expected and observed segregation for the five generations tested. There was no significant deviation from the expected 1:1 ratio.

The donor organisms for the *cry1F*, *cry34Ab1*, *cry35Ab1* and *pat* genes are soil-inhabiting bacteria. Neither of these bacteria are plant or human pathogens, and the Cry1F, Cry34Ab1, Cry35Ab1 and PAT proteins encoded by these genes do not cause disease or the production of infectious agents in plants. The synthetic, maize-optimized *cry34Ab1*, *cry35Ab1* and *pat* coding sequences were modified for optimal expression in maize, in part, by changing their codon for improved expression in maize. The promoter and terminator for the *pat* gene are derived from cauliflower mosaic virus (CaMV) which is a plant viral pathogen (Cauliflower mosaic virus causes disease primarily in cruciferous plants). The CaMV 35S promoter and terminator sequences are non-coding, regulatory sequences of known function and do not cause disease symptoms in plants nor encode for an infectious agent.

Compositional Analysis

Compositional analyses were conducted to assess whether the composition and nutrient levels in grain and forage derived from 4114 maize were comparable to those in the conventional maize variety. Compositional comparisons between 4114 maize and conventional controls were performed using the principles and analytes outlined in the OECD consensus documents for maize composition (OECD, 2002) for new varieties of maize. The comparators were non-transgenic near-isoline maize lines with about 99% genetic similarity to 4114 maize in compositional comparisons. Grain and forage samples were also collected from non-modified maize hybrids in two different experiments. Four hybrid lines were analyzed in 2003 and an additional four hybrid lines were analyzed in 2007. The conventional commercial maize hybrids selected by Pioneer for both studies were normally planted commercially in their adapted geographic region (Pioneer 2011, p. 101). A total of eight conventional maize reference varieties were included to provide data for the development of a 99% tolerance interval for each experiment. For each compositional experiment, a 99% tolerance interval was calculated. This interval is expected to contain, with 95% confidence, 99% of the values obtained from the population of commercial reference varieties. By comparison to the 99% tolerance interval, any statistically significant differences between 4114 maize and the comparators may be put into perspective, and can be assessed for biological relevance in the context of the natural variability in maize. In addition, based on published literature (e.g. Codex 1996; Codex 2005; ILSI 2006) for maize, a combined literature range has been established. The 4114 maize analyte ranges that fell within the tolerance interval and/or combined literature range for that analyte were considered to be within the range of normal variability of commercial maize hybrids.

Pioneer's compositional analyses of 4114 maize and a near-isoline control were based on forage and grain harvested from 2010 at six field locations in the U.S. and Canada (Pioneer 2011, Table 28, p. 131). Compositional analyses of grain samples included protein, fat, acid detergent fiber, neutral detergent fiber, ash, carbohydrates, fatty acid, vitamins and minerals, key anti-nutrients, and key secondary metabolites. Compositional analyses of forage samples included protein, fat, acid detergent fiber, neutral detergent fiber, ash, carbohydrates, calcium, and phosphorus.

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Pioneer also measured the concentrations of all four expressed proteins in various growth stages (Pioneer 2011, Table 8, p. 78). The Cry1F, Cry34Ab1, and Cry35Ab1 protein concentrations in 4114 maize in each tissue were compared to respective protein concentrations in 1507, 59122, and/or 1507 x 59122 maize (Pioneer 2011, Table 10-13). The results for each study on environmental effects for Cry1F and Cry34/35Ab1 are summarized in US EPA Biopesticides Registration Action Documents (BRAD) (US-EPA 2010a; US-EPA 2010b). Complete reviews of each study can be found in the individual EPA Data Evaluation Reports. Cry1F and binary Cry34Ab1/Cry35ab1 proteins have been well established (US-EPA 2010a; US-EPA 2010b; USDA-APHIS 2001; USDA-APHIS 2005). The Cry1F, Cry34Ab1, Cry35Ab1 and PAT proteins expressed in 4114 maize are generally lower than or comparable to protein expressed in previously approved 1507, 59122 and 1507 x 59122 maize lines. The only notable exceptions were concentrations of Cry1F in 4114 maize pollen. The concentration of Cry1F protein in 4114 maize pollen (35µg/g tissue dry weight) is approximately 1.5 times that of 1507 or 1507 x 59122 maize (Pioneer 2011, Table10). Based on the Expected Environmental Concentration (EEC) of Cry1F in 4114 maize pollen (35µg/g tissue dry weight), the calculated Margin of Exposure (MOE) from 4114 maize still indicates a sufficient margin of safety for the expected environmental exposure to the Cry1F protein from 4114 maize pollen (Pioneer 2011, Table 12).

Overall, a comprehensive evaluation of 4114 maize and the controls showed no biologically meaningful differences for grain and forage compositions either for major nutrients (Pioneer 2011, Table 15-19, pp. 104-117) or key anti-nutrients in maize grain (Pioneer 2011, Table 20, p. 119). The few detected differences were either exceedingly small in magnitude or the mean component values of 4114 maize and the control were within the 99% tolerance interval. Therefore, based on the data presented by Pioneer on forage and grain, it is reasonable to assume that the foods and feeds derived from 4114 maize can be considered compositionally and nutritionally equivalent to those derived from conventional maize.

Agronomic Properties

Pioneer conducted agronomic evaluations on 4114 maize and conventional and /or control maize. These evaluations are used to determine whether 4114 maize is agronomically comparable to conventional maize and they provide reasonable scientific measures as to whether 4114 maize has plant pest potential. These assessments included 14 agronomic parameters and three seed germination parameters, two pollen characteristics (shape and color), and several observations on pest response (disease incidence and insect damage) (Pioneer 2011, Table 30 and 31, pp. 135-140). The agronomic data showed no significant differences between 4114 maize and control maize. These data support Pioneer's claim that 4114 maize is agronomically comparable to conventional maize except for the intended traits and that 4114 maize does not possess characteristics that constitute a plant pest risk compared to conventional maize.

The 4114 maize and its control lines have been field tested in the U.S. and Puerto Rico since 2006. For each field trial, a plant pathologist, entomologist and plant breeder surveyed the field at least every four weeks. The observations of naturally occurring insects, diseases and any unexpected differences between 4114 maize and control lines were summarized in Table 8.2 of the Petition. APHIS has reviewed the data and found that 4114 maize did not exhibit any

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meaningful differences compared with its non-transgenic counterparts for naturally-occurring insect or disease infestations.

The data (transformation method, introduced genes and their genetic elements, compositional analysis and agronomic properties) cited by Pioneer and reviewed by APHIS indicate that 4114 maize is not biologically different from non-transgenic conventional maize lines (with the exceptions of intended introduced genetic constructs and traits) and 4114 maize is no more susceptible to pests and diseases compared to conventional maize. The introduced genetic constructs and traits (certain lepidopteran insect and corn rootworm insect resistance and tolerance to glufosinate herbicides) are not expected to alter disease and pest susceptibilities.

Potential of 4114 maize to Impact the Weediness of Other Plants with Which It can Interbreed.

In the U.S., corn is not listed as a weed (Crockett 1977; Muenscher 1980), nor is it present in the Federal Noxious Weed List (7 CFR part 360²). Furthermore, corn is grown throughout the world without any report that it is a serious weed or that it forms persistent feral populations (Gould 1968). Like many domesticated crops, corn seed from a previous year's crop can overwinter and germinate the following year. 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 because maize is incapable of sustained reproduction outside of domestic cultivation and maize are non-invasive in natural habitats (Gould 1968). Corn possesses few of the characteristics of those plants that are notably successful as weeds (Baker 1965; Keeler 1989).

APHIS assessed whether 4114 maize is any more likely to become a weed than the isogenic non-transgenic 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 4114 maize. Pioneer has been conducting agronomic evaluations in both laboratory experiments and field trials.

The germination and dormancy evaluations were tested under laboratory conditions. Seed dormancy is an important characteristic that is often associated with plants that are considered weeds (Anderson 1996). Although dormancy is not associated with modern maize cultivars, maize seed dormancy tests can be used to determine whether 4114 maize is agronomically comparable to conventional maize and determine whether 4114 maize is more likely to pose a plant pest risk when compared to conventional maize. Standardized germination assays of the Association of Official Seed Analysts (AOSA 2007) are used as a baseline to measure the germination potential. These assays evaluate various germination parameters at the optimum temperature for growth and at a few other temperature regimes to assess other seed germination properties (Pioneer 2011, Table 23, page 126). A near-isoline control (approximately 99% similar) was used for comparison and two commercial maize lines were evaluated in the study to establish a reference range for germination and dormancy. No statistically significant differences

² http://www.aphis.usda.gov/plant_health/plant_pest_info/weeds/downloads/weedlist-2010doc.pdf

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were detected. The results (Pioneer 2011, Table 24-27, pp. 127-128) support the conclusion that 4114 maize is comparable to conventional maize with respect to germination and potential dormancy and 4114 maize does not exhibit characteristics that would cause it to be weedier than the parental lines or conventional varieties.

A total of 14 different agronomic phenotypic characteristics, as well as observations for plant responses to plant-disease and plant-insect interactions were evaluated by Pioneer (Pioneer 2011, Table 29, pp. 133-134) at 15 U.S. locations and 2 Canadian locations across maize production regions in 2010 (Pioneer 2011, Table 28, page 131). At these 17 locations, the range of values for agronomic parameters was within the range of values expected for non-modified commercial maize hybrids. Agronomic characteristic evaluated (Pioneer 2011, Table 29, pp. 133-134) included: early population, seedling vigor, plant height, ear height, stalk lodging, root lodging, final population, stay green, time to silking, time to pollen shed, yield, pollen viability, disease incidence and insect damage. No differences in phenotypic characteristics that might contribute to enhance the fitness 4114 maize, including weediness traits, were identified. The mean values of these agronomic traits observed for 4114 maize fell within the range of values observed for the commercial maize varieties. Field trial data (Pioneer 2011, Table 30 and Table 31, pp. 135-140) indicated that 4114 maize does not exhibit characteristics that would cause it to be weedier than the near-isoline control and non-modified conventional maize.

In addition, agronomic field observation data showed no significant differences between 4114 maize and the non-transgenic counterparts for disease incidence and insect damage (other than resistance to the targeted lepidopteran and corn rootworm pests). The introduced traits, European corn borer, western corn rootworm resistance, and glufosinate ammonium herbicide tolerance, are not expected to cause 4114 maize to become a weed. The 1507 maize, which expresses Cry1F protein confers resistance to certain lepidopteran pests (including European corn borer) and was given non-regulated status by USDA-APHIS in 2001 (USDA-APHIS 2001). The 59122 maize, which expresses Cry34Ab1/ Cry35Ab1 protein together, confers resistance to corn rootworm pests and was also given non-regulated status by USDA-APHIS in 2005 (USDA-APHIS 2005). The PAT protein provides tolerance to glufosinate-ammonium herbicides and has been in various commercially grown crops given non-regulated status by USDA-APHIS since 1996 (e.g., Petition 96-068-01p, Petition 97-205-01p, Petition 98-238-01p, etc. found here: http://www.aphis.usda.gov/biotechnology/not_reg.html). There is no linkage between these proteins' expressions and any increased survival or overwintering capacity that would alter the prevalence of volunteer plants in subsequent growing seasons. The 1507 x 59122 maize hybrids have been on the market since 2005. In 2010, 1507 x 59122 maize was grown commercially on approximately 16% of U.S. maize acres (Pioneer 2011, page 32). After many years of commercialization , 1507 maize, 59122 maize and 1507 x 59122 maize hybrids have not exhibited characteristics that would indicate weediness. There is no reason to expect that 4114 maize would result in increased weediness, since 4114 maize contains the same Cry1F, Cry34Ab1, Cry35Ab1 and PAT proteins and will provide similar insect resistance and herbicide tolerance to that of 1507x59122 maize. The above considerations, together with the fact that the novel traits have no intended effect on weediness, leads USDA-APHIS to conclude that 4114 maize has no altered weediness potential compared to current commercialized varieties. Field evaluation of phenotypic and agronomic characteristics showed no differences relative to its

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comparator(s), and supports the conclusion that 4114 maize is not likely to have increased weediness compared to conventional maize varieties.

Potential for Gene Flow and Gene Introgression from 4114 Maize into Sexually-Compatible Relatives

APHIS evaluated the potential for gene introgression to occur from 4114 maize to sexually compatible wild relatives and considered whether such introgression would result in increased weediness. Introgression is a process whereby gene(s) successfully incorporate into the genome of a recipient plant.

Maize belongs to the grass family, *Poaceae*. The genus *Zea* has five species: *diploperennis* HH, *luxurians*, *mays*, *nicaraguensis*, and *perennis* (OGTR 2008). *Zea mays* (2n=20) has two subspecies of which *Z. mays* ssp *mays* is the only cultivated species. *Zea mays* is common in the US and is known only from cultivation. Occasionally it is found in abandoned fields or on roadsides. The closest wild relatives of maize are the teosintes which are sexually compatible with *Zea mays*. All teosinte members can be crossed with cultivated corn to produce fertile first generation hybrids (Doebley1990a; Wilkes 1967). However maize teosinte hybrids exhibit low fitness and have little impact on gene introgression in subsequent generation (Galinat, 1988). Additionally, teosintes are normally confined to the tropical and subtropical regions of Mexico, Guatemala, and Nicaragua. A fairly rare, sparsely dispersed feral population of teosinte has been reported in Florida (USDA PLANTS Database³, accessed 11/18/2011).

Maize is a predominantly an outcrossing plant species. The rate of self-pollination is 5% (Sleper and Poehlman, 2006). The short viability period of pollen grains limits the possibility of outcrossing. Since 4114 maize does not exhibit characteristics that can cause it to be any weedier 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.

Tripsacum is a genus of seven species, three of which occur in the US (Gould and Shaw 1983). Although, it is difficult, *Tripsacum* can be successfully hand crossed with maize to form hybrids. However these hybrids have a high degree of sterility (Doebley 1990a; Wilkes 1967) and are generally unstable because of differences in chromosome number and lack of pairing between chromosomes (Eubanks 1997). First generation hybrids are much less fit for survival and dissemination in the wild and typically show reduced reproductive capacity. Furthermore, gene flow from maize to *Tripsacum* is virtually impossible because of several factors including distribution, genetic incompatibility, temporal separation of flowering time, etc. (Galinat 1988).

None of the sexually compatible relatives of maize in the U.S. are considered to be weeds in the U.S. (Holm et al., 1979), therefore, the unlikely acquisition of an herbicide tolerance gene and three insect tolerance *cry* genes would not be expected to transform them into weeds. Therefore, USDA has concluded that adverse consequences of gene flow from 4114 maize to wild or weedy species in the U.S. are highly unlikely.

³ http://plants.usda.gov/java/county?state_name=Florida&statefips=12&symbol=ZEME

Potential Effects on Non-target Organisms, Including those Beneficial to Agriculture.

APHIS evaluated the potential for 4114 maize to have damaging or toxic effects directly or indirectly on non-target organisms. Non-target organisms considered were representatives of the exposed species in the agriculture environment.

For previous EPA registrations, Pioneer/DuPont submitted the Safety Assessments evaluating the effect of Cry1F, Cry34/35Ab1, and Cry1F x Cry34/35Ab1 maize events on required and voluntary host range species. EPA reviewed and concluded that the levels of Cry1F, Cry34Ab1, Cry35Ab1 and Cry1F x Cry34/35Ab1 proteins in 1507, 59122 and 1507 x 59122 maize will not pose unreasonable adverse effects to corn field flora and fauna (US-EPA, 2010a; US-EPA 2010b; US-EPA, 2010c). Available data also indicate that there should be minimal short-term accumulation of Cry proteins in agricultural soil. No evidence of synergy between the lepidopteran-active and coleopteran-active insecticidal proteins expressed by 1507 x 59122 maize was found in laboratory studies of target and non-target insects evaluated for the registration. The proteins expressed in 4114 maize are identical to those in previously approved events 1507, 59122 and 1507 x 59122 maize (Pioneer 2011, Section 2 and Appendix 2). Therefore, previous safety studies conducted in 1507 and 59122 and 1507x59122 maize should be relevant for 4114 maize.

In addition, these Cry proteins do not share any amino acid sequence similarities with known protein toxins which have adverse effects on mammals. Regarding allergenicity potential, all expressed Cry proteins originate from a non-allergenic source. Cry proteins have no sequence similarities or homology compared with known allergens; the Cry proteins will only be present at low levels in food; and the proteins expressed in 4114 are not glycosylated. The potential for any of the expressed proteins to be food allergens is minimal.

The *pat* gene comes from *Streptomyces viridochromogenes* and encodes the enzyme phosphinothricin acetyltransferase. The PAT protein homologs are likely ubiquitous in the environment. PAT protein shares no significant homology with any protein known to be toxic or allergenic (OECD 1999; ILSI 2011). A dose equivalent to 5000 mg/kg body weight was used for the PAT toxicity study. No signs of toxicity were seen in any of the groups of mice tested over a period of 14 days. Acute toxicity studies in mice show the PAT protein has no toxicity even at doses much higher than mice could encounter due to exposure to GE plants expressing the PAT protein (Herouet et al. 2005). In addition, the PAT protein is rapidly digested in experiments simulating the gastric environment (Herouet et al. 2005). Eight plant species expressing PAT proteins have been approved for environmental release in 11 different countries and have been well documented (ILSI 2011). Data from peer-reviewed literature show that the PAT protein expressed in GE plants has negligible impact on the phenotype of those plants (ILSI 2011). Since PAT protein is widespread in the environment, Canadian, Australian, Japanese and U.S. regulatory authorities have concluded that the expression of PAT protein in GE plants does not have any significant potential to adversely impact other organisms (CFIA 1995; OGTR 2003; Japanese BCH 2010; USDA APHIS 1998).

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Pioneer also assessed the non-target impact of 4114 on beneficial organisms in the corn agroecosystem. At present, APHIS is not aware of any identified significant adverse effects of Cry1F, Cry34/35Ab1 and PAT proteins on the abundance of non-target beneficial organisms in the field. Field testing reports submitted to APHIS showed minimal to undetectable changes in the beneficial insect abundance or diversity. To date, available field test data show that compared to crops treated with conventional chemical pesticides, the transgenic crops have no detrimental effect on the abundance of non-target insect populations.

The 4114 maize is comparable in agronomic characteristics and compositional characteristics to non-transgenic conventional maize except for the presence of the introduced proteins. As described earlier, the Cry1F and PAT proteins are identical to those in previously approved 1507 maize; Cry34Ab1, Cry35Ab1 and PAT are identical to those in previously approved 59122 maize and PAT protein has been approved across several crops to use commercially in U.S. since 1996. The effects of using glufosinate in 4114 (preferred alternative) is the same as for using glufosinate for 1707/59122 (no action alternative). If the effect of using glufosinate is the same in the alternatives, then no significant impact is likely due to glufosinate use. In 2010, 1507 x 59122 maize was grown on approximately 16% of U.S. maize acres. Many years of data on these proteins demonstrate the lack of toxicity to humans and animals, and the absence of adverse effects on non-target organisms and the environment.

Potential for Transfer of Genetic Information to Organisms with which 4114 maize Cannot Interbreed.

APHIS examined the potential for the new genetic material inserted into 4114 maize 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. Horizontal gene transfer and expression of DNA from a plant species to other species is highly unlikely to occur based on the following reasons.

The horizontal gene transfer (HGT) between unrelated organisms is one of the most intensively studied fields of science. Horizontal gene transfer and expression of DNA from a plant species to bacteria or animal species is unlikely to occur. A number of points support this conclusion:

1. Many genomes (or parts thereof) from bacteria that are closely associated with plants have been sequenced 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. Also, *Bacillus thuringiensis* species are generally common in soil and therefore various *cry* genes have been available for long periods of time for horizontal transfer from *Bacillus thuringiensis* species to plants or soil microorganisms and decaying plant material. Therefore the likelihood of any impact or new horizontal gene transfer that is not already capable of taking place in the soil is extremely unlikely.
2. No evidence has been identified for any mechanism by which maize genes could be transferred to humans or animals, or any evidence that such gene transfer has occurred for any plant species during evolutionary history, despite animals and humans eating large quantities of plant DNA. In cases where review of sequence data implied that horizontal gene transfer

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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).

3. 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.

4. 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 extremely unlikely (FDA 1998).

5. APHIS also considered whether horizontal transfer of DNA from 4114 maize 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 (Keese 2008). Although sequences of the Cauliflower Mosaic Virus are contained within 4114 maize, those sequences are limited to the regulatory elements. Regulatory elements such as promoters and terminators have not been implicated in viral recombination.

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 plant pest risk.

Potential Changes to Agricultural or Cultivation Practices.

APHIS considered potential impacts associated with the cultivation of European corn borer-resistant, corn rootworm-resistant and glufosinate-ammonium tolerant 4114 maize on current agricultural practices in corn and other crops.

Pioneer has provided data which indicate that 4114 maize expresses the Cry1F, Cry34Ab1 and Cry35Ab1 and PAT proteins to allow control of major corn pests and weeds. In 4114 maize, the genes for the Cry1F, Cry34Ab1, Cry35Ab1 and PAT have been integrated at a single locus in the genome; this is in contrast to the 1507X59122 maize where the insertions for the events are located at two unlinked loci. Pioneer indicates “ The option to use 4114 maize will reduce the number of breeding loci over 1507X59122 maize, thus increasing the speed at which new products will be available to growers. ... Furthermore, each trait locus must be homozygous in an inbred line and as more loci are combined, the proportion of plants that are homozygous for each locus becomes smaller, resulting in more seed discards during the breeding process.” (Pioneer 2011) Efficient breeding of multiple traits in single commercial maize, Pioneer anticipates that 4114 maize is expected to be less expensive and likely to replace commercial products containing 1507 x 59122 maize. Compared to currently-available Bt and herbicide tolerant products containing 1507 x 59122 maize, no increased use of insecticides or herbicides from the use of 4114 maize is expected.

Bt corn has been available since 1996. Planting of Bt corn grew from about 8 percent of U.S. corn acreage in 1997 to 65 percent in 2011 (USDA-ERS 2011). The NASS June Agriculture Survey (USDA-ERS 2011) indicated the increases in acreage share in recent years may be

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largely due to the commercial introduction of a new variety of Bt corn that controls corn rootworm, a pest that may be more destructive to corn yield than the European corn borer. The 4114 maize contains both rootworm and European corn borer resistance. Some reduction of chemical insecticide and herbicide use by growers is expected (vs. conventional corn). The reduced chemical pesticide use will benefit the environment directly and can mean less exposure to people who apply chemical pesticides to corn. Finally, given that 4114 maize is highly likely to constitute a replacement product for similar corn lines that are already grown on significant acres in the U.S. and which do not present plant pest risks, APHIS concludes that 4114 maize also does not present a plant pest risk.

Conclusion

APHIS has prepared the plant pest risk assessment in order to determine if 4114 maize is unlikely to pose a plant pest risk. Based on the information provided by the applicant and the lack of plant pest risk from the inserted genetic material, weedy characteristics, atypical responses to disease, insects or plant pests in the field, effects on non-targets or beneficial organisms in the agro-ecosystem, changes to agricultural practices, and horizontal gene transfer, APHIS has concluded that 4114 maize is highly unlikely to pose a plant pest risk.

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