

Plant Pest Risk Assessment for Event 3272 Corn

This plant pest risk assessment is to determine whether Event 3272 corn is unlikely to pose a plant pest risk. If APHIS determines that a genetically engineered (GE) organism is not a plant pest, then the GE organism is not subject to the regulatory requirements of 7 CFR part 340.

APHIS' authority to regulate genetically engineered organisms under the Plant Protection Act (PPA) (7 U.S.C. Sec 7701 *et seq.*) is limited to those GE organisms that are plant pests as defined under Section 403(14) of the PPA (7 U.S.C. 7702(14)).

“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.”

APHIS will use the information submitted by the applicant in its petition for nonregulated status, per § 340.6(c)(4), to determine the plant pest risk associated with the regulated article. Specifically, APHIS uses the information 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, any impacts on the weediness of any other plant with which it can interbreed, and the transfer of genetic information to organisms with which it cannot interbreed to analyze the potential plant pest risk, if any, associated with Event 3272 corn. Issues related to agricultural or cultivation practices will be considered in the Environmental Assessment for Event 3272 corn. Event 3272 corn is not genetically engineered to produce a toxin or pesticide, thus Event 3272 corn is not targeted for use against pests in corn agriculture. Thus, APHIS did not examine the effects of the regulated article on nontarget organisms. However, APHIS does examine the effects of Event 3272 corn on animals, plants, and threatened and/or endangered species (TES) species in the Environmental Assessment. APHIS has not identified any issues related to indirect plant pest effects on agricultural production caused by Event 3272 corn.

Potential impacts to be addressed in this risk assessment are those that pertain to the use of Event 3272 corn and its progeny in the absence of confinement. The genetically engineered construct inserted in Event 3272 corn was evaluated to determine if the introduced genetic sequences cause plant disease. Morphological characteristics of Event 3272 corn were analyzed to determine if this corn variety would become weedy or

invasive. The potential for gene flow to, and introgression of the genetically engineered constructs into, other corn varieties or wild relatives of corn were also evaluated to determine the potential of increased weedy or invasive characteristics in other plant species. APHIS also analyzed the propensity of Event 3272 corn to become a greater reservoir of plant pests (insects or pathogens) compared to conventional corn and potential for horizontal gene transfer.

Development of Alpha-Amylase Event 3272 Corn

Microbially-produced alpha-amylases are commonly used commercially in the starch-processing step during corn dry grind and wet milling processing. Syngenta has developed a thermostable alpha-amylase enzyme (AMY797E) expressed in Event 3272 corn grain for use in the dry grind fuel ethanol process in the U.S. The product concept of Event 3272 corn is that Event 3272 grain will serve as the source of alpha-amylase enzyme in the dry-grind ethanol process, replacing the addition of microbially-produced enzyme. Event 3272 corn will be grown using current agronomic practices, and grain expressing AMY797E alpha-amylase enzyme will be processed at the ethanol processing plant.

1. Description of inserted genetic material and potential of the material to cause plant disease.

Event 3272 corn has been genetically engineered to contain two transgenes: (1) the *amy797E* gene encoding the thermostable AMY797E alpha-amylase enzyme and (2) the *pmi* (*manA*) gene from *Escherichia coli*, which encodes the enzyme phosphomannose isomerase, used as a selectable marker. The AMY797E alpha-amylase enzyme is a chimeric, thermostable enzyme derived from three alpha-amylase genes originating from three hyperthermophilic microorganisms of the archaeal order *Thermococcales*. This enzyme was selected by Syngenta due to its increased thermostability and activity during dry grind ethanol production from corn. AMY797E is functionally similar to those thermostable, genetically engineered alpha-amylases (e.g. *Bacillus* species) currently used, and have a history of safe use in food and feed processing (Janeček et al. 1999, Lévêque et al. 2000, Pariza and Johnson 2001, Olempska-Beer et al. 2006). The expression of *amy797E* is driven by the promoter from a corn seed storage (gamma-zein) gene, which directs the accumulation of alpha-amylase in the corn kernel. The *pmi* gene is from one of the main species of bacteria living in mammal intestines, *E. coli*, and is driven by the polyubiquitin promoter from corn.

This genetic insert also contains the terminator sequences from two plant pests, cauliflower mosaic virus and *Agrobacterium tumefaciens*. Both of these sequences are well-characterized, and are non-coding regulatory regions only. These sequences will not cause Event 3272 to promote plant disease.

DNA was introduced into corn cells from a proprietary corn line using disarmed (non-plant pest causing) *Agrobacterium tumefaciens*-mediated transformation methodology with the transformation vector designated pNOV7013. 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*. Therefore, no part of the plant pest *A. tumefaciens* is remaining in Event 3272 corn due to the transformation method.

Data from Southern analyses demonstrate that Event 3272 plants: (1) contain a single copy of both *amy797E* (Figure 3-4, page 35 of petition) and *pmi* (Figure 3-6, page 36 of petition) genes, (2) contain a single copy of both the gamma-zein (Figure 3-8, page 37 of petition) and ubiquitin promoters (Figure 3-10, page 38 of petition), and (3) do not contain sequences from the transformation plasmid (pNOV7013) (Figure 3-12, page 40 of petition) that were not intended to be transferred to Event 3272 (i.e. 'backbone sequences'). DNA sequences of Event 3272 corn confirmed that the overall integrity of the intended insert and the contiguousness of the functional elements have been maintained (page 30, Figure 3-15, and page 42 of petition). Statistical analyses over multiple generations confirm that the *amy797E* gene is stably integrated and is inherited over generations in the expected fashion (Table 3-2, page 31 of petition). Therefore, only the expected genetic material was stably inserted into Event 3272 corn, and there is no expectation that plant disease will result due to the genetic construct in Event 3272 corn.

2. Potential impacts based on the relative weediness and/or invasiveness of Event 3272 corn

APHIS assessed whether Event 3272 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 Event 3272 corn.

In the U.S., corn is not listed as a weed in the major weed references (Muenscher 1980, Holm et al. 1991, Holm et al. 1997) nor is it listed as a noxious weed species by the U.S. 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 because 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 and rarely reappear in a second season. Corn also possesses few of the characteristics of plants that are notably successful weeds (Baker 1965, Keeler 1989).

Syngenta conducted agronomic field trials of Event 3272 corn at a total of 25 locations in the U.S. corn belt during the 2003 and 2004 growing seasons. Field trial data (Tables 5-3, 5-4, 5-5, 5-6, 5-7, 5-8, Appendix 4 of petition) indicated that Event 3272 corn does not exhibit characteristics that would cause it to be weedier than the parental corn line.

Growth habitat was not remarkably different between Event 3272 corn and the control hybrid (Table 5-3 of petition). Two measures of late season integrity of the corn plant, late season intactness and push test scores, were slightly depressed in Event 3272 plants; however a third integrity measure, percent broken stalks, was elevated in Event 3272 plants compared to the non-transgenic control hybrids. Thus, overall, growth habitat characteristics did not differ in Event 3272 corn compared to the hybrid controls.

Vegetative vigor assessments (Table 5-4 of petition) conducted by Syngenta indicate that early emergence vigor, early growth rating and ear height characteristics were all similar between Event 3272 corn and the control hybrids. Plant height was found to be significantly shorter in Event 3272 corn compared to the hybrid control plants. However, plant height reduction is unlikely to be associated with a trend toward increasing weediness.

Syngenta also evaluated the reproductive characteristics (Table 5-5 of petition) of Event 3272 corn. The percentage of barren plants was slightly elevated in Event 3272 corn compared to the nontransgenic hybrid controls, and this was the only reproductive variable that differed between the two plant types. In terms of weediness, an increase in barren plants in Event 3272 corn would not increase the risk of weediness because it does not indicate a potential increase in reproductive output.

There was no increase in weediness potential as measured by differences in primary dormancy (germination potential) or secondary dormancy (overwintering ability) (Table 5-8 of petition). There was a slight decrease in the ability of Event 3272 seeds to survive the 'winter conditions' test (5°C) compared to the nontransgenic control plants. However, a decrease in seed survival would not correspond to an increase in secondary dormancy and increased weediness. In addition, there were no changes to abiotic stress tolerance (as measured by early root lodging, late root lodging, and percent snapped plants) (Table 5-3 of petition).

The introduced traits, increased alpha-amylase accumulation in the grain and expression of PMI, are not expected to cause Event 3272 corn to become a weed or improve the ability of this corn variety to survive without human intervention. Nor is there any foreseeable reason to conclude that these two genes would affect this variety's survival in the wild. None of the characteristics of weeds described by Baker (Baker 1965) involves increased alpha-amylase or PMI levels, and there is no reason to expect that these traits would result in increased weediness. Event 3272 corn is unchanged in its susceptibility to injury by commercially available herbicides, and thus could be chemically-controlled. Therefore, there is no selective advantage to corn containing AMY797E or PMI compared to conventional corn, and there is no increased potential for weediness or invasiveness from Event 3272 corn.

3. Potential impacts from gene flow and gene introgression from Event 3272 corn into its sexually-compatible relatives.

Zea mays L. subsp. *mays* is a member of the *Maydeae* tribe of the grass family, *Poaceae*. It is a monoecious annual 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 (OECD 2003).

In assessing the risk of gene introgression from Event 3272 corn into its sexually compatible relatives, APHIS considered two primary issues: 1) the potential for gene flow and introgression, and 2) the potential impact of introgression.

APHIS evaluated the potential for gene introgression to occur from Event 3272 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 Jr. et al. 2003). Based upon currently available data, there have been a relatively low number of confirmed cases of introgression (Stewart Jr. 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. A few isolated populations of annual (*Zea mexicana*) and perennial (*Zea perennis*) teosinte have been reported to exist in the past in Alabama, Florida, South Carolina, and Maryland (USDA-NRCS 2007), but are likely no longer in existence (US-EPA 2000), or are small isolated occurrences. None of these teosinte species has been shown to be an aggressive weed in its native or introduced habitats. The Mexican and Central America teosinte populations primarily exist within and around cultivated corn 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). 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 F₁ hybrids (Wilkes 1967, Doebley 1990a). 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).

Nevertheless, in the wild, introgressive hybridization from maize to teosinte is currently limited, in part, by several factors including distribution, differing degrees of genetic incompatibility, temporal separation in flowering time, differences in developmental morphology, variation in dissemination methods, and disparities in dormancy (Galinat

1988, Doebley 1990a, 1990b). 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 corn in the Americas over thousands of years, but corn and teosinte maintain distinct genetic constitutions despite sporadic introgression (Doebley 1990a). The potential for gene introgression from Event 3272 corn into teosinte would increase if varieties are developed and approved for cultivation in locations where these teosintes are located. However, hybridization in nature is extremely unlikely because the distributions of teosinte and *Z. mays* do not overlap (<http://www.maizegenetics.net/index.php?page=domestication/taxonomydistribution.html>), and because of differences in developmental morphology and reproductive timing between the two species. Additionally first-generation corn-teosinte hybrids are generally less fit for survival and dissemination, and they show substantially reduced reproductive capacity. Therefore, it is very unlikely that gene introgression into a wild corn relative will occur. Moreover, since Event 3272 corn does not exhibit characteristics to 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 corn varieties.

The genus *Tripsacum* contains up to 16 recognized species, most of which are native to Mexico, Central and South America, but three exist or have existed as wild and/or cultivated species in the U.S. (Hitchcock 1971, USDA-NRCS 2007). Though many of these species occur where corn might be cultivated, gene introgression from Event 3272 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 is able to withstand even the mildest winters (Beadle 1980, Galinat 1988). Furthermore, none of the sexually compatible relatives of corn in the U.S. is considered to be a weed in the U.S. (Holm et al. 1997). AMY797E and PMI do not confer a selective advantage to plants that contain these genes. Thus, the likelihood of introgression is extremely low. In the highly unlikely event that introgression to a wild relative would occur, acquisition of the *amy797E* or *pmi* gene would not be expected to transform the wild relative into a weed.

4. Potential of Event 3272 corn to harbor plant pests (insects and disease)

The data submitted by Syngenta indicated no significant differences between Event 3272 corn and the non-transgenic counterparts for disease (as measured by northern corn leaf blight and southern corn leaf blight observations, and gray leaf spot rating) and pest susceptibility (as measured by European corn borer damage) (Table 5-7 of petition). The data presented in the petition indicated no difference in compositional and nutritional quality of Event 3272 corn compared to conventional corn, apart from the presence of AMY797E and PMI. Although some of the variables measured by the applicant showed statistically significant differences between Event 3272 corn and the nontransgenic hybrid controls (Tables 6-1 to 6-6, pages 70-76), none of the values for the forage and grain composition characteristics was outside the range of natural variability of conventional corn as found in 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 Event 3272 is not biologically different than conventional corn, and is thus susceptible to the same pest population as conventional corn. Additionally, Event 3272 corn is similarly affected by typical plant diseases found in corn, and does not harbor an altered pest or pathogen community compared to other corn varieties.

5. Transfer of genetic information to organisms with which it cannot interbreed

Horizontal gene transfer and expression of DNA from a plant species to bacteria 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>). 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 Event 3272 corn. Due to the lack of plant pest risk from the inserted genetic material, the lack of weediness characteristics of Event 3272 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 Event 3272 corn is unlikely to pose a plant pest risk.

Moreover, APHIS has also concluded that the existence of the alpha-amylase enzyme, by itself, does not constitute a plant pest in Event 3272 corn. The alpha-amylase enzyme engineered into Event 3272 corn is not one of the organisms listed in the statutory definition of a plant pest. Enzymes such as alpha-amylase are proteins that catalyze chemical reactions. Enzymes are not “living.” Thus, enzymes cannot be plant pests because they are not living and cannot be a “living stage” of any of the organisms (“articles”) listed in the PPA’s definition of a plant pest in subparagraphs (A) through (G) of 7 U.S.C. 7702(14). Likewise, the Event 3272 corn alpha-amylase enzyme also cannot be a living stage of any article similar to or allied with any of the articles specified in subparagraphs (A) through (G), and thus does not fall within the statutory definition of a plant pest as listed in subparagraph (H) of the PPA’s plant pest definition (i.e., “Any article similar to or allied with any of the articles specified in the preceding

subparagraphs”). APHIS has determined that the alpha-amylase enzyme engineered into Event 3272 corn is not a plant pest because the alpha-amylase enzyme in Event 3272 corn is not living and thus cannot itself be a living stage of any organism listed in the PPA’s plant pest definition.

APHIS has determined that neither Event 3272 corn, nor the alpha-amylase enzyme in Event 3272 corn, is a “living stage” of any of the organisms (articles) listed in subsections A-H of the PPA’s plant pest definition. Moreover, neither Event 3272 corn nor the alpha-amylase enzyme in Event 3272 corn is an article that harbors plant pests.

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