PLANT PEST RISK ASSESSMENT FOR MON 87460 CORN

Monsanto Company has petitioned the Animal and Plant Health Inspection Service (APHIS) of the United States Department of Agriculture for a determination that genetically engineered (GE) corn (*Zea mays*) event MON 87460 (APHIS number 09-055-01p-a1) is unlikely to pose a plant pest risk and, therefore, should no longer be a regulated article under APHIS' 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 2000[1]. This plant pest risk assessment was conducted to determine whether MON 87460 corn is unlikely to pose a plant pest risk.

History of Development of MON 87460 Drought-Tolerant Corn

Corn is the second major food and feed crop in world. Recently corn is also considered as a major biofuel crop in the U.S.; therefore, it is expected that U.S. corn cultivation may continue to increase in the coming years (USDA 2007). Conventional breeding methods have made enormous progress in corn crop improvement (Sprague and Dudley 1988). Despite those improvements the demand for corn far exceeds the current production level, leading to a worldwide grain shortage.

As with many high yielding modern crop plants, drought is one of the major limiting factors in corn that prevents realization of optimum grain yield (Heisey and Edmeades 1999), and that problem may become a frequent feature across U.S. farmlands and elsewhere in the future. Abiotic stress is the primary cause of crop loss worldwide, reducing average yields for most major crop plants by more than 50% (Boyer 1982; Bray et al. 2000). In North America alone, it is estimated that 40% of annual crop losses are due to sub-optimal water availability (Boyer 1982). Although some of the recent corn cultivars are bred to exhibit some degree of drought tolerance (Byrne et al. 1995; Tollenaar and Wu 1999; Bängiger et al. 2006), the current corn gene pool and conventional breeding techniques may not be able to bring a significant improvement for drought tolerance in future corn cultivars. In this regard, new biotechniques combined with conventional breeding methods offer potential new tools and avenues to harness drought tolerant gene from different sources to increase corn yield stability under water-limited conditions (Bänziger and Araus 2007).

Drought conditions vary in nature, severity, and impact (Jacobsen and Shaw 1989; Close et al. 1993; Campos et al. 2006). While extreme drought conditions prevent normal growth and development of plants leading to complete crop failure, suboptimal soil water at key plant growth stages can also substantially reduce crop yield. Drought tolerant crops are designed to escape water limitation through both genetic and agronomic manipulations. The strength of drought tolerance is a relative quantification measured over existing cultivars, which varies greatly among crop plants. Under no circumstances, however, it is likely that crop improvement alone can mitigate all economic losses under water-limited conditions; rather it has the potential to play a key role in minimizing crop loss in drought-prone areas (Heisey and Morris 2006).

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[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."

Monsanto has developed MON 87460 corn event (hereafter referred to as MON 87460) that is expected to reduce yield loss under water-limited conditions compared to conventional corn. The ability to withstand the water limitation in MON 87460 comes from an introduced gene called cold shock protein B (CSPB) derived from the bacterium *Bacillus subtilis*. The CSPB is a RNA chaperone. RNA chaperone**s** are ubiquitous and abundant proteins found in all living organisms and viruses. RNA tends to be kinetically trapped in misfolded forms, and RNA binding proteins, acting as chaperones, can resolve these structures, ensuring accessibility for its biological function. In bacteria, RNA chaperones are believed to play a general role in sustaining growth by favoring active transcription, translation, and/or ribosome assembly (Castiglioni et al. 2008). In bacteria, the CSPB protein helps preserve normal cellular functions during certain stresses by binding cellular RNA and unfolding nontranslatable secondary structures affecting RNA stability and translation. As in bacteria, the CSPB protein in MON 87460 binds RNA and appears to help maintain plant cellular functions.

Data submitted by Monsanto suggest that MON 87460 reduces yield loss, primarily through increased kernel number per ear, under water-limited conditions by minimizing the effect of water limitation on photosynthesis, stomatal conductance, and carbon fixation. On a plant level, corn yield losses associated with drought stress occur as a result of reduced synchrony between anthesis and silking, embryo loss, and/or reduced grain filling in viable kernels (Claassen and Shaw 1970; Boyer and Westgate 2004; Campos et al. 2006). Studies on conventional germplasm with enhanced drought tolerance show that yield improvements are attained through improvements in all of these endpoints (Bolanos et al. 1993: Bolanos and Edmeads 1996: Barker et al. 2005). Therefore, the enhanced yield stability of MON 87460 under water-limited conditions, conferred by the expressed CSPB protein, appears to be the result of improvements in the natural stress response mechanisms over conventional corn.

MON 87460 corn has been field tested under APHIS regulations since 2002 and Monsanto provided data in the petition for field trials completed prior to the petition submission. Because MON 87460 reduces yield loss under water-limited conditions, Monsanto designed field studies to collect relevant data across a broad range of soil moisture (well watered and water-limited conditions) and environmental conditions relevant to where commercial production would be expected. Data submitted by Monsanto shows that under well-watered conditions, grain yield for MON 87460 is not significantly different from conventional corn, yet MON87460 experienced reduced yield loss when water stress was around 20% less than normal. However, like conventional corn, MON 87460 is still subject to yield loss under severe water-limited conditions, particularly during flowering and grainfill periods when corn yield potential is most sensitive to water stress. In the following paragraphs APHIS BRS summarizes its plant pest risks assessment of MON 87460 because of inserted genetic elements and/or their products.

Description of the Inserted Genetic Material

MON 87460 was developed through a plant pathogenic bacterium *Agrobacterium tumefaciens* mediated transformation of corn line LH59 embryos (Armstrong and Phillips 1988). However, the *A. tumefaciens* strain, ABI, that was used to develop MON 87460, was made non pathogenic by removing the pathogenic sequences present in Ti (tumor inducing) plasmid originally present in *A. tumefaciens* (Koncz and Schell 1986). The disarmed *A. tumefaciens* harbors a binary

plasmid vector PV-ZMAP595 (Monsanto 2010, Figure III-1, p. 97). This vector was approximately 9.4 kb and contained a single T-DNA delineated by left and right border regions with in which there were two expression cassettes: a *cspB* gene expression cassette, which contained coding sequence for CSPB protein, and a neomycin phosphotransferase II (*nptII*) expression cassette, which contained coding sequence for the NPTII protein.

The *cspB* expression cassette consisted of the following genetic elements (Monsanto 2010, Table IV-1, p. 100):

- Promoter, leader, and intron from the rice (*Oryza sativa*) actin gene, *Ract1* (McElroy et al. 1990).
- Full length coding sequences of Cold Shock Protein B (*cspB*) gene from the bacterium, *Bacillus subtilis* (Willimsky et al. 1992).
- 3' nontranslated terminator sequence of the *transcript* 7 gene from *Agrobacterium tumefaciens* that directs polyadenylation (Dhaese et al. 1983).

The *nptII* expression cassette consisted of the following genetic elements (Monsanto 2010, Table IV-1, p. 100):

- *loxP* sequence from *Bacteriophage P1* for the recombination site recognized by Cre recombinase (Russell et al. 1992). Cre-Lox recombination provides a way to knockout genes.
- P-*35S* promoter for the 35S RNA of the Cauliflower mosaic virus (Odell et al. 1985).
- CS-*nptII* coding sequence from *Tn5* (Beck et al. 1982) in *Escherichia coli* encoding neomycin and kanamycin resistance (Fraley et al. 1983) that was used as a selectable marker during transformation selection.
- T-*nos* 3' nontranslated sequence of the *nopaline synthase* (NOS) gene from *A. tumefaciens.* This sequence terminates gene expression by a polyadenylation site (Depicker et al. 1982).
- *loxP* sequence from *Bacteriophage P1* for the recombination site recognized by Cre recombinase (Russell et al. 1992). The *loxP* sites were inserted to facilitate the potential excision of the *nptII* cassette using CRE recombinase.

In addition to the above-mentioned genetic elements, the insert also contained noncoding intervening sequences of 1-73 base pair length and right and left border sequences that were used during DNA cloning.

Monsanto 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, PV-ZMAp595 (Monsanto 2010, Figure V-5, p. 117; Figure V-15, p. 134),
- the DNA inserted into the corn genome is present at a single locus and contains one functional copy of *cspB* and *nptII* genes (Monsanto 2010, Figure V-4, p. 116; Figure V-14, p. 133),

- the sequence and organization of inserted genes in MON 87460 are identical to their original sequences and arrangements in the donor plasmid PV-ZMAp595 (based on DNA sequence analysis),
- no novel open reading frames were created that spanned either the 5′ or 3′ junctions between the T-DNA and corn genomic sequences, and
- the stability of introduced genes was demonstrated by the presence of introduced genes via Southern blot fingerprint of MON 87460 (Monsanto 2010, Figures V-14 and V-15, p. 133-134) for seven generations tested (Monsanto 2010, Figure V-13, p. 132) in the breeding history. The stability was further confirmed by the Mendelian inheritance of the T-DNA in MON 87460 (Monsanto 2010, Table V-3, p. 136).

Two minor changes occurred during the MON 87460 transformation event; one in the inserted genetic element and one to the adjacent corn genomic DNA. A part (733 base pairs) of the promoter, rice actin gene (*Ract1*), did not get incorporated into the transformed plant. According to Monsanto, this rearrangement probably resulted from double-strand break repair mechanisms in the plant during the *Agrobacterium*-mediated transformation process (Salomon and Puchta 1998). Furthermore, analysis of the T-DNA insertion site indicated that a 22 base pair length of genomic DNA got deleted at the insert-to-plant DNA junction in MON 87460. Despite these minor genetic sequence modifications in MON 87460, neither the stability analysis of the inserted genetic elements over seven generations (Monsanto 2010, Figures V-14 and V-15, p. 133-134; Monsanto 2010, Table V-3, p. 136), nor the forage and grain compositional assessment (p. 151-174 in Monsanto 2010) showed any biologically meaningful differences between MON 87460 and conventional corn control except for the drought tolerance traits.

PLANT PEST RISK ASSESSMENT

MON 87460 corn was produced by transformation of corn tissue using *A. tumefaciens* to introduce two genes: a gene (*cspB*) that confers drought tolerance and a gene (*nptII*) that facilitates selection of transformed plants. Because *A. tumefaciens* is a plant pest and some of the regulatory sequences (35S Promoter sequence from Cauliflower mosaic virus and nopaline synthase terminator sequence from *A. tumefaciens*) 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.

Potential impacts to be addressed in this risk assessment are those that pertain to the use of MON 87460 corn and its progeny in the absence of confinement. APHIS utilizes data and information submitted by the applicant, in addition to current literature, to determine if MON 87460 is unlikely to pose a plant pest risk. If APHIS determines that a GE organism is unlikely to pose a plant pest risk, then APHIS has no regulatory authority over that organism.

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, expression of the gene product, changes to plant metabolism, disease and pest susceptibilities, impacts on the weediness of any other plant with which it can interbreed, weediness of the regulated article, and impacts on nontarget organisms. Issues related to

agricultural or cultivation practices are discussed in the Environmental Assessment prepared by APHIS for MON 87460 corn.

Potential Impacts of Genetic Modifications on Altered Disease and Pest Susceptibilities

USDA-APHIS assessed whether MON 87460 corn is likely to have significantly increased disease and pest susceptibility. This assessment encompasses a thorough consideration of introduced traits, their impact on agronomic traits and plant composition, and interactions with pests and diseases.

Introduced Traits

MON 87460 contains drought tolerance traits from the introduced gene product CSPB protein and a selectable marker gene product NPTII protein. The CSPB protein in MON 87460 belongs to the cold-shock protein (CSP) family and is identical in amino acid sequence to the native CSPB protein produced in *B. subtilis* (Willimsky et al., 1992) with the exception of one amino acid change in the second position from leucine to valine. This amino acid change was implemented by Monsanto to facilitate the assembly of the plasmid vector PV-ZMAP595 for plant transformation. The structure of CSPB is well characterized (Schindelin et al. 1993; Schindelin et al. 1994) and in bacteria CSPs are composed of 67-73 amino acids (Graumann et al. 1997). The CSPB protein in MON 87460 consists of 66 amino acids (Monsanto 2010, Figure IV-1, p. 102). Although Figure IV-1 shows 67 amino acids for the CSPB protein in MON 87460, which were predicted based on *cspB* coding sequence, following translation, however, the N-terminal methionine is cleaved by methionine aminopeptidase leaving 66 amino acids in the final CSPB protein in MON 87460 (Monsanto 2010, Appendix B, p. 283-284). CSPs are a group of small proteins that contain a highly conserved RNA-binding sequence identified as a cold shock domain (CSD). Under environmental stress, bacterial CSD-containing proteins have been shown to bind many types of RNA (Cristofari and Darlix, 2002), leading to sustainable translation, maintenance of mRNA levels, and improved cellular function. Similar to bacteria, CSD-containing proteins in plants also bind RNA, unfold RNA secondary structures caused by environmental stress, and help maintain cellular functions under stress. These plant CSD proteins share a high level of similarity to the bacterial CSPs and have been shown to share *in vitro* and *in vivo* functions with bacterial CSPs (Karlson and Imai 2003; Kim et al. 2007; Nakaminami et al. 2005 and 2006; Chaikam and Karlson 2008; Fusaro et al. 2007). As with bacterial and other plant CSD-containing proteins, the CSPB protein from *B.subtilis*, which is expressed in MON 87460, binds RNA, unfolds RNA secondary structures, and accumulates in actively growing tissues (Monsanto 2010, Figures I-7 – I-9). CSPB accumulation is highest in rapidly growing areas of the leaf and seedling and tends to decline as the tissue matures. CSPB concentrations tend to increase over time in developing ears and decline over time in silks. Likewise, CSPB concentrations increase over time in immature tassels and either remain the same or decline in pollen. Taken together, the data on RNA binding, CSPB accumulation and CSPB localization in MON 87460 are consistent with the pattern of RNA binding, accumulation, localization, and functions described for plant CSD-containing proteins (Fusaro et al. 2007; Sasaki et al. 2007; Chaikam and Karlson 2008).

Monsanto did not observe any abnormal or altered mechanisms of plant response to drought stress in transgenic CSPB-containing corn plants. Greenhouse experiments were conducted with CSPB events that are genetically and phenotypically similar to MON 87460 to evaluate if these

characteristics had been altered when compared with controls. The plants containing CSPB protein show broadly the same relative water content (Monsanto 2010, Table I-4), leaf water potential (Monsanto 2010, Figure I-22), and leaf osmotic potential (Monsanto 2010, Figure I-23) as the control plants. CSPB-containing plants accumulate similar levels of abscisic acid (ABA) (Monsanto 2010, Figure I-24) and osmotically active solutes (sucrose, fructose, glucose, choline, proline, glycine betaine) as observed in controls (Monsanto 2010, Figures I-25 - 28). Results from these experiments suggest that common mechanisms of plant response to drought stress are not altered in transgenic CSPB-containing corn plants. Thus, like endogenous CSD proteins found in plants, the CSPB protein in MON 87460 interacts with RNA, accumulates, and localizes to rapidly growing tissues and in developing reproductive organs, thereby helping to maintain normal cellular function in those tissues critical to yield under water-limited conditions. Monsanto observed that the major component contributing to the improved yield of MON 87460 under water-limited conditions was the increased number of kernels per plant (Monsanto 2010, Tables I-1 & I-2, p. 46-47), which is consistent with the current understanding of the effect of drought stress on corn yield potential (Barker et al. 2005).

NPTII protein is well characterized (Fuchs et al. 1993), and in MON 87460 NPTII protein consists of 264 amino acids (Monsanto 2010, Figure IV-2, p. 102). The NPTII protein in MON 87460 confers resistance to kanamycin, so as to facilitate the selection of transformed plants (Bevan et al. 1983). The effect of NPTII protein on plant growth and development has been extensively evaluated through several lines of experimental evidence, and several products containing NPTII have been approved by regulatory agencies on a global basis(e.g. so far NPTII was used as a selectable marker in 28 petitions deregulated by APHIS BRS; http://www.isb.vt.edu/cfdocs/biopetitions1.cfm). NPTII is the most commonly used antibiotic resistance marker in several commercially grown biotechnology-derived crops including YieldGard® Rootworm corn (MON 863), Bollgard® cotton (MON 531), Bollgard®II cotton (MON 15985), and Roundup Ready cotton (MON 1445). NPTII protein was never found to facilitate plant pest characteristics to plants. Based on the known functions and mechanisms of these proteins (summarized in Monsanto 2010), neither of these proteins are expected to directly alter susceptibility to plant pathogens.

Compositional Analysis

Compositional comparisons between MON 87460 and a conventional control were performed using the principles and analytes outlined in the OECD consensus documents for corn composition (OECD 2002 and 2006). The control substance was a conventional corn hybrid with genetic background similar to MON 87460. The investigation also included 15 other conventional commercial corn hybrids (Monsanto 2010, Appendix Tables E-1 and E-2, p. 311). The commercial reference hybrids selected by Monsanto for each study were adapted to the geographic region in which they were grown with selections based on agronomic characteristics such as relative maturity and drought tolerance ratings. The rationale for the use of commercial references in various experiments was to provide data for the development of a 99% tolerance interval for each variable analyzed. This interval is expected to contain, with 95% confidence, 99% of the values obtained from the population of commercial corn highlighting the existing natural variability in the commercial corn under cultivation. The knowledge of existing natural variability was used to draw biological meanings into those statistically significant differences between MON 87460 and controls. This comparative evaluation also considered natural ranges

in corn component levels published in the literature or in the International Life Sciences Institute Crop Composition Database (ILSI 2006).

Monsanto's compositional analyses of MON 87460 were based on forage and grain harvested from two different growing seasons, the first during 2006 in the U.S. conducted under typical agronomic practices and water conditions, and the second during 2006/2007 in Chile under well-watered and water-limited conditions. Components evaluated in samples harvested from both studies included (1) moisture, protein, carbohydrates by calculation, fat, fiber, and ash in a proximate analysis, (2) essential macro- and micro-nutrients in a nutritional analysis, and (3) known endogenous toxicants and anti-nutrients. A supplementary analysis of secondary metabolites associated with stress tolerance was also conducted by Monsanto for samples from Chile in 2006 and 2007 (see Appendix E for details). In total, there were 434 comparisons made for compositional analyses (7 sets of comparisons x 53 components from grain) + (7 sets of comparisons x 9 components from forage).

Overall, a comprehensive evaluation of MON 87460 and the control showed no biologically meaningful differences for grain and forage compositions either for major nutrients (Monsanto 2010, Table VII-2, p. 157-158; Table VII-3, p. 164; Table VII-5, p. 168) or for secondary metabolites (Monsanto 2010, Table VII-6, p. 171; Monsanto 2010, Table VII-7, p. 173). The few detected differences were either exceedingly small in magnitude or the mean component values of MON 87460 and the control were within the 99% tolerance interval. Therefore, based on the data presented by Monsanto on forage and grain, it is reasonable to assume that the foods and feeds derived from MON 87460 can be considered compositionally equivalent to those derived from conventional corn.

Agronomic Properties

Monsanto evaluated phenotypic and agronomic characteristics of MON 87460 in a comparative manner to assess plant pest potential (OECD, 1993). These assessments included 14 plant growth and development characteristics, five seed germination parameters, two pollen characteristics, several observations on plant-insect and plant-disease interactions, and plant responses to abiotic stressors (Monsanto 2010, Table VIII-1, p. 178). Monsanto provided phenotypic and agronomic data (see Section VIII. Phenotypic, Agronomic, and Environmental Interactions Assessment, p. 176-231) supporting their claims that MON 87460 is similar to unmodified control corn cultivar except for the intended drought tolerant trait and that MON 87460 does not possess characteristics that would confer a plant pest risk compared to conventional corn. Detailed agronomic and phenotypic characteristics are discussed further in relevant sections of this document, while the observed disease and pest susceptibility of MON 87460 is discussed in the following paragraph.

Monsanto used well-established qualitative or quantitative techniques to measure field trials for damage due to diseases (Anthracnose, Ear rot, Kernel rot, Eyespot, *Fusarium*, Gray leaf spot, Gray mold, Leaf blight, Maize dwarf mosaic virus, Northern leaf spot, *Pythium*, Rust, Seedling blight, Smut, Southern leaf blight, Stalk rot, Stewarts wilt, and Yellow leaf blight), insect pests (Aphids, Leafhoppers, Armyworms, Corn earworms, European corn borers, Southwestern corn borers, Grasshoppers, Corn rootworms, Flea beetles, Japanese beetles, Grape colaspis, Wireworms, White grubs, Spider mites, Seedcorn maggots) for two years conducted across

different agroecological conditions. The data submitted by Monsanto indicated no meaningful differences between MON 87460 corn and the non-transgenic counterparts for diseases (Monsanto 2010, Table H-2, p. 432), or insect pests (Monsanto 2010, Tables H-3 to H-7, p. 433-441; Table H-4, p. 434: Tables H-5 to H-6, p. p. 435-438). Thus MON 87460 corn is expected to be susceptible to the same plant pathogens and insect pests as conventional corn.

Monsanto's aforementioned data (description of the inserted genetic elements, expression of the gene product, compositional analysis, and agronomic and phenotypic observations) indicates that MON 87460 corn is not biologically different from conventional corn (with the exception of the CSPB3 and NPTII proteins), and MON87460 is no more susceptible to pests and diseases compared to conventional corn cultivars.

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 (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).

Corn is a monoecious species with separate male and female inflorescences that enable cross pollination. Corn is predominantly a wind pollinated outcrosser with occasional bee visitation for pollen. Bees rarely visit female inflorescences (silk). Researchers recognize that: (i) the percent gene flow will vary by population, hybrid or inbred, (ii) the level of gene flow decreases with greater distance between the source and recipient plants; (iii) environmental factors affect the level of gene flow, (iv) corn pollen is viable for a relatively short period of time under field conditions, (v) corn produces ample pollen over an extended period of time, and (vi) corn is not pollinated by insects (pollinating insects, especially bees, are occasional visitors to the tassels but rarely visit silks of corn) (Jemison and Vayda, 2000; Luna et al., 2001).

APHIS evaluated the potential for gene introgression to occur from MON 87460 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 several members of the genus *Zea*, and to a much lesser degree with members of the genus *Tripsacum* (OECD 2003; Monsanto 2010, Table II-1, p. 82). 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 (Wilkes 1967; Matsuoke et al. 2002; Fukunaga et al. 2005). Corn and annual teosinte (*Zea mays* subsp. *mexicana*) are genetically compatible, wind-pollinated, and may hybridize when in close proximity to each other, e.g., in areas of Mexico and Guatemala. While some teosinte may be considered weeds in certain instances, they are also used by some farmers for breeding improved maize (Sánchez and Ruiz 1997 and references therein). Teosinte is described as being susceptible to many of the same pests and diseases that attack cultivated corn (Sánchez and 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). Genetically based cross-incompatibility mechanisms are known to reduce hybrids between corn and teosinte when corn is the pollen source (Baltazar et al. 2005; Kermicle and Evans 2005), which thus acts as a significant constraint to introgression.

Teosinte is not present in the U.S. other than as an occasional botanical garden specimen and a few small feral populations of *Zea mexicana* in Florida, Alabama, and Maryland and *Zea perennis* in South Carolina (http://plants.usda.gov). Introgression of genes from corn into teosinte or *Tripsacum* species has not been described to occur in nature in the U.S. Furthermore, MON 87460 is unlikely to outcross with sexually compatible species in the U.S. as differences in factors such as flowering time, geographical separation, and development factors make natural crosses in the U.S. highly unlikely.

Corn is also distantly related to the species in the genus *Tripsacum*, which contains up to 16 recognized species (Monsanto 2010, Table II-1, p. 82), 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 (http://plants.usda.gov). In contrast with corn and teosinte which easily hybridize under certain conditions, special techniques are required to hybridize corn and *Tripsacum* and the offspring of the cross show varying levels of sterility (Galinat 1988; Mangelsdorf 1974; Russell and Hallauer 1980). 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 MON 87460 corn and wild relatives, the transgenes of MON 87460 corn are unlikely to transform corn wild relatives into more weedy species. Based on the data presented in the petition, MON 87460 corn does not exhibit characteristics that cause it to be any weedier than other cultivated corn (see the section "Potential Impacts Based on the Relative Weediness of MON 87460 Corn" 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, MON 87460 corn is unlikely to adversely impact sexually compatible wild relatives or their weediness characters.

Potential Impacts Based on the Relative Weediness of MON 87460 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. Corn is poorly suited to survive without human assistance and is not capable of surviving as a weed (Baker 1965; Keeler 1989; Galinat 1988). 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 subsequent years. Corn does not possess suit of traits that are characteristics of successful weeds (Baker 1965; Keeler 1989).

APHIS assessed whether MON 87460 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 MON 87460 corn evaluated under field conditions, including potential weedines traits such as seed dormancy and germination, rate of growth and development, flowering, seed dispersal, seed yield, and persistence of free living populations outside cultivation. Monsanto collected phenotypic and agronomic field data from field trials conducted at 27 U.S. locations during the 2006 and 2007 growing seasons that included diverse agroecological regions representative of the major corn-growing areas of the upper mid-west in the U.S. Likewise, Monsanto also collected complimentary data from four field trials from Chile (Monsanto 2010, Table VIII-2, p. 189). In addition, data on abiotic stress tolerance from greenhouse and growth chamber assays were used to characterize the extent of stress tolerance imparted by the insertion of the *cspB* gene and determine whether any potential changes in tolerance enhanced weediness potential in MON 87460.

For the majority of the traits assessed, there were no statistically significant differences between MON 87460 and nontransgenic control (Monsanto 2010, Tables VIII-4 to VIII-12, p. 192-204; Tables VIII-14 to VIII-16, p. 208-210). No biologically meaningful differences were detected in the germination and dormancy of seed from MON 87460. In particular, the absence of hard seed supports a conclusion of no increased weediness potential of MON 87460 compared to conventional corn for germination and dormancy characteristics (Monsanto 2010, Table VIII-14, p. 208). No differences were detected in pollen morphology or viability between MON 87460 and the control (Monsanto 2010, Table VIII-15 & VIII-16, p. 209-210). Results from the genotype-environmental interaction assessments also support the conclusion that MON 87460 has no increased susceptibility or tolerance to specific diseases, arthropods, or abiotic stressors, with the exception of drought. Development of deep root system is typically one of the agronomic traits that has potential to enhance weediness in the natural environment. However that may not be the case with corn, as inbred lines with poor early root development exhibited higher yields under drought stress than ones with accelerated early development of roots (Bruce et al. 2002). It has been reasoned that the improved performance of corn cultivars to drought stress probably comes from better water use efficiency (Messmer et al. 2009). Finally, MON 87460 was not altered in its ability to volunteer in cultivated fields or survive in areas not managed for agricultural production compared to conventional corn.

Based on the agronomic field data and literature survey concerning corn's weediness potential, MON 87460 corn is unlikely to persist as a troublesome weed or to impact on current weed management practices. Furthermore, extensive post-harvest monitoring of field trial plots planted with MON 87460 under USDA-APHIS notifications (Monsanto 2010, Appendix N, p. 519-523) did not reveal any differences in survivability or persistence relative to other corn. These data suggest that MON 87460 is no more likely to become a weed than conventional corn.

Potential Impacts on Nontarget Organisms, Including Beneficial Organisms

Monsanto is in consultation with FDA about food and feed derived from corn event MON 87460 and has submitted a comprehensive assessment of food and feed safety data on CSPB and NPTII proteins of MON 87460 to FDA. Monsanto considered the following factors to establish food and feed safety of introduced gene products.

- The donor organism of the CSPB protein, *B. subtilis*, is not pathogenic, is oftenused as a food additive, is present in many fermented foods, and has a history of safe consumption. In 1999, FDA designated enzyme preparations from this organism as GRAS (generally recognized as safe, FDA, 1999).
- The *B. subtilis* CSPB shares a high percent of identity with CSPs present in other bacterial species broadly used by the food industry and with CSD-containing proteins in plant species used as a food. Many foods prepared with the help of *B. subtilis* have been consumed for a long time with no documented history of any adverse effects to human health. CSPB protein present in MON 87460 shares amino acid identity to other naturally occurring CSD-containing proteins found in food and feed products. The amino acid identity of CSPB ranges from 35% to 98.5% across different plant and bacterial species. The CSPB protein is homologous to the CSP proteins found in the genera *Lactobacillus*, *Lactococcus*, *Bifidobacterium*, and *E. coli*, which are normally present in gastrointestinal flora and, therefore, considered to be safe.
- A dietary safety assessment conducted to evaluate the risks to humans and animals from the CSPB and NPTII proteins present in the foods and feeds derived from MON 87460 did not cause any observed adverse effects even at the highest tested dose levels.
- CSPB protein represents no more than 0.00007% of the total protein in the grain of MON 87460. Digestive fate experiments conducted with the CSPB protein demonstrated that the full-length protein is rapidly digested in simulated gastric fluid, a characteristic shared among many proteins with a history of safe consumption. Proteins that are rapidly digestible in mammalian gastrointestinal systems are unlikely to be allergens when consumed.
- CSPB and NPTII proteins do not share any amino acid sequence similarities with known allergens, gliadins, glutenins, or protein toxins which have adverse effects to mammals.
- The safety of the NPTII protein and its donor organism, *E. coli*, have been recognized by regulatory agencies and well documented. All existing data suggest that the NPTII protein represents a negligible hazard to human health and is safe for consumption.

The introduced gene products of MON 87460 do not contain pesticidal activity and are not aimed at any target organisms as a control measure. As mentioned earlier, MON 87460 is found to be safe for human and animal consumption. Therefore, Monsanto assessed the nontarget impact of MON 87460 on all those exposed organisms (e.g. pests, diseases, and beneficial arthropods) in the corn agroecosystem.

During the U.S. and Chile phenotypic field studies at 31 locations in 2006 and 2007, each field site was rated at four time intervals during the season for specific insects (pest and non-pests), and diseases (Monsanto 2010, Section VIII.F.1, p. 211-212) to evaluate whether the plant-disease or plant-insect interactions of MON 87460 were altered compared to commercial corn. Out of nearly 388 pest and non-pest arthropod evaluations, only two differences were observed between MON 87460 and the control. Grasshopper damage was

lower for MON 87460 compared to the control and European corn borer damage was higher for MON 87460 compared to the control. These differences were within the range of the references observed for corn cultivars. Likewise, out of the more than 425 disease stressor observations, no differences were detected between MON 87460 and the control. These results support the conclusion that no biologically meaningful interactions were observed with the species exposed to MON 87460 and that MON 87460 is not different in its environmental interactions relative to other corn.

Potential Impacts from Transferring Genetic Information from MON 87460 Corn to Organisms with which It cannot Interbreed

APHIS examined the potential for the new genetic material inserted into MON 87460 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 since 1940, and the issue gained extra attention with the release of transgenic plants into the environment (Droge et al. 1998). Horizontal gene transfer has been implicated as a major contributor to the spread of antibiotic resistance amongst pathogenic bacteria and the emergence of increased virulence in bacteria, eukaryotes, and viruses; and has contributed to major transitions in evolution.

Potential for Horizontal Gene Transfer to Bacteria, Fungi, OR Viruses

The MON 87460 contains two genes and two noncoding regulatory sequences from bacteria. 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), so far there are no reports of horizontal gene transfer 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 MON 87460 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; Kaneko et al. 2002; Wood et al. 2001). 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, 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). APHIS also considered whether horizontal transfer of DNA from MON 87460 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). There are two noncoding regulatory sequences (35S and *LoxP*) from virus present in MON 87460 corn. These two sequences have not been implicated in viral recombination raising safety concerns (Hull et al. 2000). 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 MON 87460 corn. Due to the lack of plant pest risk from the inserted genetic material, the lack of atypical responses to disease or plant pests in the field, the lack of weediness characteristics of MON 87460 corn, 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 MON 87460 corn is unlikely to pose a plant pest risk.

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