

Monsanto Petition (10-281-01p) for Determination of Non-regulated Status of MON 87427

**OECD Unique Identifier:
MON-87427-7**

Plant Pest Risk Assessment

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A. Introduction

Monsanto Company has petitioned APHIS (APHIS number 10-281-01p) for a determination that genetically engineered (GE) corn (*Zea mays* L. subsp. *mays*) event MON 87427 is unlikely to pose a plant pest risk (Monsanto, 2010) and, therefore, should no longer be a regulated article under APHIS' 7 Code of Federal Regulations (CFR) part 340. APHIS administers 7 CFR part 340 under the authority of the plant pest provisions of the Plant Protection Act of 2000¹. This plant pest risk assessment was conducted to determine if MON 87427 is unlikely to pose a plant pest risk.

MON 87427 utilizes a specific promoter and intron combination (*e35S-hsp70*) to drive CP4 EPSPS protein expression in vegetative and female reproductive tissues, conferring tolerance to glyphosate herbicide in the leaves, stalk, and root tissues and tissues that develop into seed or grain and silks. This specific promoter and intron combination also results in limited or no production of CP4 EPSPS protein in two key male reproductive tissues: pollen microspores which develop into pollen grains, and tapetum cells that supply nutrients to the pollen.

Potential impacts to be addressed in this risk assessment are those that pertain to the use of MON 87427 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 87427 is unlikely to pose a plant pest risk. If APHIS determines that a GE organism is not a plant pest, then APHIS has no regulatory authority over that organism.

Potential impacts in this Plant Pest Risk Assessment are those that pertain to plant pest risk characteristics. APHIS regulation 7 CFR 340.6(c) specifies the information needed for consideration in a petition for nonregulated status. APHIS evaluates information submitted by the applicant related to plant pest risk characteristics, disease and pest susceptibilities, expression of the gene product, new enzymes, or changes to plant metabolism, weediness of the regulated article, impacts on the weediness of any other plant with which it can interbreed, and transfer of genetic information to organisms with which it cannot interbreed.

The Environmental Assessment (EA) for this petition considers whether agricultural or cultivation practices for MON 87427 may result in impacts on the environment. A thorough assessment of potential effects on non-target and beneficial organisms, and threatened and endangered species is included in the Environmental Assessment.

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."

B. Development of MON 87427

Since the 1930s, corn productivity in the U.S. has been greatly enhanced by the use of hybrid corn seed (Griliches 1960; Perez-Prat and van Lookeren Campagne 2002; Slepser 2006). Corn hybrids are characterized by increased resistance to diseases and enhanced agronomic characteristics compared with the parental lines (Brewbaker 1964; Duvick 2001). The production of hybrid corn seed involves a cross between two inbred lines², where the pollen from the tassel/male parent is used to fertilize the ear/female parent. Because corn is mostly self-pollinated (OECD 2003), hybrid corn seed is typically produced by the removal of male flowers (tassels) from the female parent either mechanically or by hand. However, mechanical detasseling can result in up to 40 percent reduction in seed yield when compared to that of hand detasseling treatments (Wych 1988). In addition, female plants may be blown over by a storm and escape detasseling, allowing secondary tassels to develop after completion of the manual detasseling. In either case, some of the female plants will be self-pollinated resulting in seed of the female inbred being harvested along with the hybrid seed.

As an alternative to tassel removal, numerous genetic strategies have been attempted to achieve male sterility³ (Skibbe 2005). Over 40 genetic elements are associated with male sterility. Some of these elements are located on the nuclear chromosomes while others are located on the mitochondrial chromosomes (Skibbe 2005). A major drawback to male sterile genetic approaches is the difficulty in generating male inbred lines because no functional pollen is produced in a male sterile plant line.

Monsanto has developed a novel method (Monsanto 2010, pages 33-36) in which EPSPS protein expression occurs in vegetative and female reproductive tissues thereby conferring tolerance to glyphosate herbicide in the leaves, stalk, and root tissues and tissues that develop into seed or grain and silks. As detailed in the petition, the specific promoter and intron combination results in limited or no production of EPSPS protein in two key male reproductive tissues: pollen microspores which develop into pollen grains, and tapetum cells that supply nutrients to the pollen, which essentially limits or eliminates the production of viable pollen in the male plant (Huang 2009; Monsanto 2010).

Monsanto has completed a food/feed safety consultation on MON 87427 with the Food and Drug Administration (FDA) (Monsanto 2010, p. 28). See announcement on <http://www.accessdata.fda.gov/scripts/fcn/fcnDetailNavigation.cfm?rpt=bioListing&id=90>. They have also submitted information to the Environmental Protection Agency (EPA) requesting herbicide label changes to reflect the new use patterns of glyphosate that this product would require (Monsanto 2010, p. 28). These changes are now approved and reflected on a supplemental label.

² Inbred lines are populations of identical or nearly identical plants used as stocks for the creation of hybrid lines.

³ Male sterility - the inability to produce functional pollen.

C. Expression of the Gene Product and Changes to Plant Metabolism

MON 87427 was developed through *Agrobacterium*-mediated transformation of maize immature embryos from line LH198 × HiII utilizing PV-ZMAP1043. The LH198 inbred line was released in 1992 by Holden's Foundation Seeds, Inc of Williamsburg Iowa. LH198 is an inbred related to the stiff-stalk family and was derived from the cross (LH132 × B84) × LH132. LH132 is also a Holden's Foundation Seed inbred and B84 is an inbred released by Iowa State University. The HiII inbred germplasm was specifically developed for use in maize transformation and is publicly available from the Maize Genetics Stock Center. The HiII germplasm was derived from the cross between two Stiff Stalk inbreds B73 and A188 (Armstrong et al. 1991).

Plasmid Vector PV-ZMAP1043 - (Monsanto 2010, p. 33):

- PV-ZMAP1043 is approximately 8.9 kb and contains one T-DNA that is delineated by Left and Right Border sequences. The T-DNA contains one expression cassette consisting of the *cp4 epsps* coding sequence under the regulation of the *e35S* promoter, the *hsp70* intron, the *CTP2* targeting sequence, and a *nos* 3' non-translated region.
- The backbone region of PV-ZMAP1043, located outside of the T-DNA, contains two origins of replication for maintenance of the plasmid vector in bacteria (*ori V*, *ori-pBR322*), a bacterial selectable marker gene (*aadA*), and a coding sequence for repressor of primer protein for maintenance of plasmid vector copy number in *E. coli* (*rop*).

The *cp4 epsps* Coding Sequence and the CP4 EPSPS Protein (T-DNA) – (Monsanto 2010, p. 35-36)

- The *cp4 epsps* expression cassette encodes a 47.6 kDa CP4 EPSPS protein consisting of a single polypeptide of 455 amino acids. The *cp4 epsps* coding sequence is the codon optimized coding sequence of the *aroA* gene from *Agrobacterium* sp. Strain CP4 encoding CP4 EPSPS. The CP4 EPSPS protein is similar and functionally identical to endogenous plant EPSPS enzymes, but has a much reduced affinity for glyphosate.
- The *cp4 epsps* coding sequence in MON 87427 is under the regulation of the *e35S* promoter, the *hsp70* intron, the *CTP2* targeting sequence, and a *nos* 3' non-translated region. The *e35S* promoter, which directs transcription in plant cells, contains the duplicated enhancer region from the cauliflower mosaic virus (CaMV) 35S RNA promoter. The *hsp70* intron is the first intron from the maize heat shock protein 70 gene. The *CTP2* targeting sequence is the targeting sequence from the *ShkG* gene encoding the chloroplast transit peptide region of *Arabidopsis thaliana* EPSPS that directs transport of the CP4 EPSPS protein to the chloroplast. The *nos* 3' non-translated region is the 3' non-translated region of the *nopaline synthase* (*nos*) gene from *Agrobacterium tumefaciens* that terminates transcription and directs polyadenylation.

- PV-ZMAP1043 contains Right Border and Left Border regions that were derived from *Agrobacterium tumefaciens*. The border regions each contain a 24-25 bp nick site that is the site of DNA exchange during transformation. The border regions separate the T-DNA from the plasmid backbone region and are involved in their efficient transfer into the maize genome.
- Genetic elements that exist outside of the T-DNA borders are those that are essential for the maintenance or selection of PV-ZMAP1043 in bacteria. The origin of replication *ori V* is required for the maintenance of the plasmid in *Agrobacterium* and is derived from the broad host plasmid RK2. The origin of replication *ori-pBR322* is required for the maintenance of the plasmid in *Escherichia coli* and is derived from the plasmid vector pBR322. Coding sequence *rop* is the coding sequence of the repressor of primer protein and is necessary for the maintenance of plasmid copy number in *E. coli*. The selectable marker *aadA* is a bacterial promoter and coding sequence for an enzyme from transposon Tn7 that confers spectinomycin and streptomycin resistance in *E. coli* and *Agrobacterium* during molecular cloning. Because these elements are outside the border regions, they are not expected to be transferred into the maize genome. The absence of the backbone sequence in MON 87427 was confirmed by Southern blot analyses.

Data from Southern blot analyses demonstrate that MON 87427 contains a single copy of the *cp4 epsps* expression cassette, stably integrated at a single locus, stably inherited over multiple generations following Mendelian principles. No plasmid vector backbone sequences were detected in MON 87427. DNA sequencing analyses determined the exact sequence of the inserted DNA and allowed a comparison to the T-DNA sequence in the plasmid vector confirming that only the expected sequences were integrated.

The data presented in the petition indicate no difference in compositional and nutritional quality between MON 87427 and conventional corn, apart from the presence of the *cp4 epsps* expression cassette (Monsanto 2010). None of the values for seed composition characteristics were outside the range of natural variability of conventional corn (Monsanto 2010, Appendix E). Therefore, the composition of MON 87427 is not biologically different than conventional corn (with the exception of the presence of a specific promoter and intron combination resulting in limited or no production of CP4 EPSPS protein in two key male reproductive tissues: pollen microspores which develop into pollen grains, and tapetum cells that supply nutrients to the pollen).

D. Potential Impacts on Disease and Pest Susceptibilities

APHIS assessed whether MON 87427 is likely to have significantly altered disease and pest susceptibility. This assessment encompassed a thorough consideration of the introduced trait and disease and pest susceptibility data from MON 87427 field trials.

MON 87427 has been field tested in the U.S. since 2005 (Monsanto 2010, Appendix A). Agronomic data was collected at 16 different locations in 2008 that provided a range of environmental conditions representative of where MON 87427 is expected to be grown

(Monsanto 2010, Table G-2). Monsanto routinely monitors their corn field trials for 24 common corn disease agents (Monsanto 2010, table G-5), at least 22 insect pest species (table G-6-G8) and the presence of at least 8 different groups of functional beneficial arthropods (table G-9). The data submitted by Monsanto indicate no meaningful differences between MON 87427 and non-transgenic counterparts for disease, insect damage and the presence of beneficials (Monsanto 2010, Appendix G).

Furthermore, DNA sequences derived from plant pests that were incorporated in MON 87427 did not result in the production of infectious agents or disease symptoms in field trials with the plants, and so it is unlikely that MON 87427 could pose a plant pest risk. The description of the genetic modifications, including genetic elements, expression of the gene product and their functions for MON 87427 has been summarized above.

The use of disarmed *Agrobacterium* for transforming MON 87427 is unlikely to propagate infectious agents. Transformed plants used in the generation of MON 87427 were treated with an antibiotic to eliminate the *Agrobacterium* from plant culture using well known protocols (Monsanto 2010).

APHIS considered whether corn constituents that differed between MON 87427 corn and other corn varieties might be sufficient to lead to increased susceptibility to pathogens or pests. However, as discussed above (p.4), there are no indications of differences in compositional and nutritional quality between MON 87427 and conventional corn, apart from the presence of the *cp4 epsps* expression cassette. Based on the known functions and mechanisms of actions of the new protein (summarized in Monsanto 2010), this protein is not expected to directly alter susceptibility to plant pathogens. Thus MON 87427 is expected to be susceptible to the same plant pathogens as conventional corn.

Corn is not a plant pest in the United States⁴, and the introduced DNA in MON 87427 is unlikely to pose a plant pest risk. Based on the analysis of genetic modifications and their functions and field testing data submitted by the petitioner, APHIS concludes that there are no significant differences between MON 87427 corn and the non-transgenic counterparts relative to pest and disease susceptibility.

E. Potential Impacts on Nontarget Organisms Beneficial to Agriculture

Monsanto provided a brief summary of an evaluation of the abundance of key beneficial arthropods in corn fields where MON 87427 and its comparator were simultaneously grown. An assessment of spiders, big-eyed bugs, brown and green lacewings, ladybird beetles, micro Hymenoptera, nabids and *Orius*, indicate that the abundance of these beneficial arthropods is not different between the two corn production systems (Monsanto 2010, pages 294-297). Moreover, EPSPS protein has a long history of safe use in these plants. In 2011, glyphosate-resistant varieties were grown on approximately 93 percent of soybean acres, 78 percent of upland cotton acres, and 70 percent of corn acres in the United States (USDA-ERS 2011). The EPSPS proteins have been commercialized since 1994 and are present in crops which are grown on millions of acres in the U.S.

⁴ http://www.aphis.usda.gov/plant_health/plant_pest_info/weeds/downloads/weedlist2006.pdf

every year. At present, APHIS is not aware of any identified significant adverse effects of EPSPS proteins on the abundance of non-target organisms in the field.

F. Potential for Enhanced Weediness of MON 87427

Corn possesses few of the characteristics of those plants that are notably successful as weeds (Keeler 1989). 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 360)⁵. 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. For instance, the appearance of corn seedlings in soybean fields following a corn crop is a common occurrence. Manual or chemical measures are often applied to remove these volunteers, but the plants that are not removed do not typically result in feral populations in following years.

APHIS assessed whether MON 87427 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 MON 87427 under field conditions presented in the 10-281-01p petition. Monsanto has conducted over 600 agronomic field trials of MON 87427 since 2005 in the U.S. corn belt at different locations (Monsanto 2010, Appendix A). Agronomic data were collected from MON 87427 and its control counterparts in 16 locations that provide a range of environmental conditions representative of where the MON 87427 is expected to be grown for seed production (Monsanto 2010, Appendix G). Field trial data (Monsanto 2010, pp. 282-296) indicate that MON 87427 does not exhibit characteristics that would cause it to be weedier than the parental corn line. No differences in phenotypic characteristics that might contribute to enhanced weediness were observed between MON 87427 and control lines for the wide range of phenotypic endpoints assessed in these field trials or in greenhouse or laboratory experiments. There was no increase in weediness potential as measured by differences observed in the field for germination, seedling vigor, days to 50% pollen shed, days to 50% silking, stay green, ear height, plant height, dropped ears, stalk lodging, root lodging, final stand count, grain moisture, test weight and yield (Monsanto 2010, table G3). None of the measured attributes showed any differences relative to its comparator, suggesting that MON 87427 is not weedier than current corn cultivars.

These results on growth characteristics, seed production and germination indicate that MON 87427 is not significantly different from its comparators. There is no indication that MON 87427 possesses a selective advantage that would result in increased weediness. MON 87427 lacks the ability to persist as a troublesome weed, and there would be no significant impact on current weed management practices for corn cultivation.

⁵ http://www.aphis.usda.gov/plant_health/plant_pest_info/weeds/downloads/weedlist.pdf

G. Potential Impacts on the Weediness of Any Other Plants with which It Can Interbreed

Gene flow from crops to wild relatives may have the potential to enhance the weediness of wild relatives, as observed in rice, sorghum, sunflower, and a few other cultivated plants (Ellstrand 1999). 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 (Soltis 1993; Rieseberg 1997) and even in existing floras, the occurrence of hybridization or introgression is reported to be widespread (Stace 1987; Rieseberg 1993). It has been a common practice by plant breeders to artificially introgress traits from wild relatives into crop plants to develop new cultivars.

APHIS evaluated the potential for gene introgression to occur from MON 87427 to sexually compatible wild relatives and considered whether such introgression would result in increased weediness. Cultivated corn is sexually compatible with other members of the genus *Zea*, and to a much lesser degree with members of the genus *Tripsacum* (OECD 2003). The closest wild relatives of corn, a number of *Zea* species referred to as teosinte, are normally confined to the tropical and subtropical regions of Mexico, Guatemala, and Nicaragua. In the U.S. a fairly rare, sparsely dispersed feral population of teosinte has been reported in Florida (USDA Plant database⁶).

The genus *Tripsacum* contains up to 16 recognized species, most of which are native to Mexico, Central and South America. Three of these species (*T. dactyloides*, *T. floridatum*, and *T. lanceolatum*) exist as wild and/or cultivated species in the continental U.S. and two taxa (*T. fasciculatum* and *T. latifolium*) also occur in Puerto Rico (USDA PLANTS Database, accessed 9/28/11). Though many of these species occur where corn might be cultivated, gene introgression from MON 87427 under natural conditions is highly unlikely. Hybrids of *Tripsacum* species with *Zea* are difficult to obtain outside of a laboratory and are often sterile or have greatly reduced fertility and none of them typically withstand even the mildest winters (Galinat 1988; Magelsdorf 1939). Furthermore, none of the sexually compatible relatives of corn in the U.S. are considered to be weeds in the U.S. (Holm 1979). Therefore, even in those instances of incidental gene flow between MON 87427 and wild relatives, the transgenes of MON 87427 are unlikely to transform corn wild relatives into more weedy species.

Introgression of genes from corn into teosinte or *Tripsacum* species has not been described to occur in nature in the U.S. While some teosinte may be considered weeds in certain instances, they are also used by some farmers for breeding improved maize (Sánchez 1997 and references therein). Teosinte is described as being susceptible to many of the same pests and diseases that attack cultivated corn (Sánchez 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

⁶ http://plants.usda.gov/java/county?state_name=Florida&statefips=12&symbol=ZEME

and 1990b; Galinat 1988; Ellstrand 2007). First-generation hybrids are generally less fit for survival and dissemination in the wild and show substantially reduced reproductive capacity, which thus acts as a significant constraint to introgression. Even if gene flow to a wild relative of corn did occur, an engineered trait that does not offer any adaptive advantage will probably not persist in the weed population (Ellstrand 1990). MON 87427 contains a gene that results in the no production of CP4 EPSPS protein in two key male reproductive tissues. This gene would not be expected to confer a selective advantage if gene flow were to occur. Data included in the petition demonstrated that there were no significant differences in viability and diameter of pollen collected from MON 87427 plants and near-isoline reference plants (Monsanto 2010, table VII-7); therefore, the outcrossing rate of MON 87427 is not expected to be any different from other corn. 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. Therefore, USDA has determined that any adverse consequences of gene flow from MON 87427 to wild or weedy species in the U.S. are highly unlikely.

H. Potential Changes to Agriculture or Cultivation Practices

Corn hybrid seed production involves a cross between two inbred lines to fertilize the female parent. Hybridization is mostly achieved by the removal of male flowers from the female parent either mechanically or by hand, a labor-intensive effort that rarely achieve 100% detasseling resulting in hybrid seed yield reduction and the possibility of undesired genetic mixing, resulting in seed of the female inbred line being harvested along with the hybrid seed. The method described for MON 87427 (Monsanto 2010, pages 33-36) confers tolerance to glyphosate herbicide in the leaves, stalk, and root tissues and tissues that develop into seed or grain and silks, except in the male corn parts (pollen microspores which develop into pollen grains, and tapetum cells that supply nutrients to the pollen), where limited or no production of EPSPS protein is achieved, which essentially limits or eliminates the production of viable pollen in the male plant (Huang 2009; Monsanto 2010). This effective technique has the potential of reducing the need of eliminating detasseling by hand or machine, utilizing the herbicide glyphosate sprayed over the corn plants. Corn hybrid seed production is relatively small (0.17%) compared with corn grain production (Monsanto 2010, page 305).

I. Potential Impacts from Transfer of Genetic Information to Organism with which MON 87427 Cannot Interbreed

APHIS assessed whether horizontal gene transfer might occur between MON 87427 corn and inserted genes with other organisms. However, such transfer and expression of DNA from a plant species to bacteria is unlikely to occur (Keese 2008). First, many bacteria (or parts thereof) that are closely associated with plants have been sequenced, including *Agrobacterium* and *Rhizobium* (Kaneko 2000; Kaneko 2002; Wood 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 were inferred to have occurred on an evolutionary time scale on the order of millions of years (Brown 2003; Koonin 2001). Third, FDA has evaluated horizontal gene transfer

following plant transformation with 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 (FDA 1998). Therefore, APHIS concludes that horizontal gene transfer is unlikely to occur and thus poses no significant environmental or plant pest risk.

J. Conclusion

APHIS has prepared this plant pest risk assessment in order to determine if event MON 87427 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 or plant pests in the field, effects on non-targets or beneficial organisms in the agro-ecosystem, and horizontal gene transfer, APHIS has concluded that event MON 87427 is unlikely to pose a plant pest risk.

K. References

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