Plant Pest Risk Assessment for Monsanto MON 87701 Soybeans

Monsanto Company (referred to hereafter as Monsanto) has petitioned APHIS (APHIS number 09-082-01p) for a determination that genetically engineered (GE) soybean (*Glycine max*) event MON 87701 is unlikely to pose a plant pest risk 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 2000[1]. This plant pest risk assessment was conducted to determine if MON 87701 is unlikely to pose a plant pest risk.

MON 87701 was produced by using *Agrobacterium*-mediated transformation (Monsanto, 2010). Because *Agrobacterium tumefaciens* is a plant pest, this soy 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 87701 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 87701 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.

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 will evaluate 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, any 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 considered whether agricultural or cultivation practices for MON 87701 may result in impacts on the environment. A thorough assessment of the effects of the determination on non-target and beneficial organisms, and threatened and endangered species is included in the EA.

Development of Lepidopteran Resistant Soybean Event MON 87701

MON 87701 soybean expresses an insecticidal protein, Cry1Ac, and was developed for the South American soybean market (Monsanto, 2010). In this region, the lepidopteran pest, *Epinotia aporema*, causes severe economic damage through eating of soybean plants (Higley and Boethel, 1994). Because it bores into the stem, larvae are protected from insecticidal sprays. Control of these insects requires high levels of systemic

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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."

insecticide treatment. To be effective, the application of these insecticides needs to be carefully timed (Higley and Boethel, 1994).

Cry1Ac is a lepidopteran-specific (e.g., *E. aporema*) insecticide derived from the soil bacterium, *Bacillus thuringiensis* (Bt). This protein does not affect other orders of insects or animals (van Frankenhuyzen, 2009). Although initially developed for the South American soybean market, United States (U.S.) growers may eventually adopt MON 87701 for commercial production if Monsanto obtains appropriate registrations from the EPA (Monsanto, 2010). Currently, MON 87701 has only received U.S. Environmental Protection Agency (EPA) approval for breeding and seed multiplication activities for a total of 15,000 acres in Georgia, South Carolina, North Carolina, Virginia, and Maryland with no more than 1,000 acres per county per year. This type of EPA registration precludes commercial sale of MON 87701 in the U.S. (US-EPA, 2010).

Soybean growers in the U.S., the world's largest exporter of this commodity, suffer 0.001% yield loss due to insect pests (Heatherly et al., 2009). Insect pests in the U.S. that could be controlled by MON87701 include: soybean looper (*Pseudoplusia includens),* corn earworm / bollworm (*Helicoverpa zea*), fall armyworm (*Spodoptera frugiperda*), green cloverworm (*Plathypena scabra*), velvetbean caterpillar (*Anticarsia gemmatalis*), lesser cornstalk borer (*Elasmopalpus lignosellus*), beet armyworm (*Spodoptera exigua*), and yellow stripe armyworm (*S. ornithogalli*) (Way, 1994)

Crystal (Cry) proteins derived from *Bacillus thuringiensis* (*Bt*) have a long history of safe use without adverse human health or environmental effects, and provide an option for the control of lepidopteran insect pests (Ferré and Van Rie, 2002). *Bt*-expressing soybean is a new commodity not commercially registered in the U.S. However, *Bt*-cotton and *Bt*-corn, that also express Cry proteins, have a long history of safe use without adverse human health or environmental effects and provide an option for the control of lepidopteran insect pests (Glare and O'Callaghan, 2000; NAS, 2004; Romeis et al., 2008).

As additional information, tolerance exemptions and conditional pesticide registrations have been granted for the plant-incorporated protectant Cry1Ac in Bollgard® and Bollgard II® cottons, GE crops that express 100% amino acid sequence identity as MON 87701 (Monsanto, 2010). MON 87701 is within the scope of the U.S. Food and Drug Administration (FDA) policy statement concerning regulation of products derived from new plant varieties, including those produced through genetic engineering. The Monsanto Company initiated the consultation process with FDA for the commercial distribution of MON 87701 and submitted a safety and nutritional assessment of food and feed derived from MON 87701 to the FDA on May 28, 2009 (BNF No. 000119) (FDA, 2010a). FDA evaluated the submission and responded to the developer by letter on August 18, 2010 (FDA, 2010b). Based on the information the Monsanto Company submitted, and as of August 5, 2010, FDA has no further questions regarding MON 87701 soybean.

Description of the Modification

MON 87701 was developed through *Agrobacterium*-mediated transformation of soybean meristem tissue utilizing transformation vector, PV-GMIR9 (Monsanto, 2010 page 38) of soybean variety A5547, a type V maturity group soybean (Monsanto, 2010 page 37). The PV-GMIR9 vector used for the transformation of soybean to produce MON 87701 is approximately 15.5 kb and contains two T-DNAs. Each of the two T-DNAs contains a single expression cassette. The first TDNA (designated as T-DNA I) contains the *cry1Ac* expression cassette, which results in the expression of Cry1Ac protein. The *cry1Ac* expression cassette contains the *cry1Ac* coding sequence under the regulation of the *RbcS4* promoter and leader, *CTP1* chloroplast targeting sequence, and the *7S'* 3' non-translated sequence. The second TDNA (designated as T-DNA II) contains the *cp4 epsps* gene expression cassette. The *cp4epsps* expression cassette contains the *cp4 epsps* coding sequence under the regulation of the *FMV* promoter, the *shkG* leader, the *CTP2* chloroplast targeting sequence and the *E9* 3' non-translated sequence.

The backbone region outside of the T-DNAs contains two origins of replication for maintenance of plasmid 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 copy number in *E. coli* (*rop*). A description of the genetic elements in PV-GMIR9 is provided in Table IV-1 of the petition.

Transformed plants were self-pollinated. The unlinked insertions of T-DNA I (*cry1Ac* gene expression cassette) and T-DNA II (*cp4 epsps* gene expression cassette) segregated in the resulting progeny. A non-lethal dose of glyphosate herbicide was applied to these plants. The plants with minor injury were selected for further analyses, whereas plants showing no injury, i.e., containing T-DNA II (*cp4 epsps* gene expression cassette), were eliminated from subsequent development. Subsequently, plants containing only a single T-DNA I (*cry1Ac* gene cassette) were identified and selected by a combination of analytical techniques, including ELISA and TaqMan PCR analysis. MON 87701 was selected from these plants. (See section III of Monsanto, 2010 for a more detailed description.)

MON 87701 has been genetically engineered to contain one transgene construct[2]: a *RbcS4* promoter, a full-length *cry1Ac* gene, a *CTP*1 chloroplast targeting sequence, and a 7S α' 3' non-translated region.

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[2] The *cp4 epsps* gene from *Arabidopsis thaliana* (Monsanto, 2010, page 45), encoding for the selectable marker glyphosate tolerance, was also incorporated as part of the initial transformation process. However, the transformation process resulted in the introduction of the *cry1Ac* gene and the *cp4 epsps* gene into two different loci. The presence of the *cp4 epsps* gene allowed for selection of transformed cells containing *cry1Ac* gene in the presence of glyphosate. As part of the breeding process to develop MON 87701, the *cp4 epsps* gene was bred out, and is not present in MON 87701 (Monsanto, 2010 Figure IV-1 and table IV-1).

- The *RbcS4* promoter and 5' non-translated region from the ribulose 1,5-bisphosphate carboxylase small subunit 1A transit peptide of *Arabidopsis thaliana* (Krebbers et al., 1988).
- *CTP*1 targeting sequence encoding the transit peptide of the Arabidopsis RbcS4 encoding small subunit 1A transit peptide, from Arabidopsis thaliana, present to direct the cry1Ac protein to the chloroplast (Krebbers et al., 1988).
- *cry1Ac*, a codon-modified coding sequence of the Cry1Ac protein of *Bacillus thuringiensis* (Fischhoff and Perlak, 1996) [3]. The Cry1Ac protein is toxic to certain lepidopteran species.
- 7Sα', the 3' region of the *Sphas*1 gene of Glycine max encoding the 7S α' seed storage protein, b-conglycinin, including 35 nucleotides of the carboxyl terminal b-conglycinin coding region with the termination codon and the polyadenylation sequence (Schuler et al., 1982). The element functions to terminate transcription and direct polyadenylation of the mRNA.
- The right and left border regions of T-DNA from *Agrobacterium tumefaciens* nopaline Ti-plasmid.
- Intervening sequence used for cloning.

Data from Southern analyses demonstrate that MON 87701 plants contain: (1) a single copy of *cry1Ac* (Monsanto, 2010, Figures V-2 to V-7, pages 58-65); (2) no sequences from the transformation plasmid (PV-GMIR9) (Monsanto, 2010) (*i.e.*'backbone sequences'); (3) no sequences from T-DNA II. (Monsanto, 2010, Figure V-7). Analysis of DNA sequences of MON 87701 confirmed that the overall integrity of the intended insert and the contiguousness of the functional elements have been maintained (Monsanto, 2010, Response Appendix 1). Statistical analyses over multiple generations confirm that the *cry1Ac* gene is stably integrated and is inherited over generations in the expected fashion (Monsanto, 2010, Figures V-8 and V-9).

Potential of MON 87701 to Become Invasive and/or a Weed

Soybeans are not considered a weed in the U.S. (Holm, 1977; Holm, 1997; Muenscher, 1980.; Reed and Hughes, 1977), and are not listed as a Federal noxious weed (7 CFR part 360[4]). APHIS assessed whether MON 87701 is any more likely to become a weed than the nontransgenic comparator, or other cultivated soybeans. The assessment encompassed a thorough consideration of the basic biology of soybeans and an evaluation of the unique

[3]-MON 87701 expresses the Cry1Ac protein, an insecticidal protein from *Bacillus thuringiensis* subsp. *kurstaki*, which provides resistance to certain lepidopteran pests. The Cry1Ac protein expressed in MON 87701 shares >99% amino acid identity with Cry1Ac from *B. thuringiensis* (Bt) subsp. *kurstaki* and 100% amino acid sequence identity with the Cry1Ac protein present in Bollgard cotton, with the exception of four additional amino acids at the N-terminus of the MON 87701-produced Cry1Ac protein. These four amino acids are derived from the chloroplast targeting sequence.

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

characteristics of MON 87701. APHIS analyzed the field test reports and other data included in the petition, as well as data from scientific literature on the establishment, reproduction, and dispersal processes of soybeans as measures of invasiveness and weediness and the effectiveness of Cry1Ac-expressing soybeans.

Monsanto provided measures of plant growth (Monsanto, 2010, pages 92-108) including; plant growth and development characteristics, seed germination parameters, pollen characteristics, and observations for plant-insect and plant-disease interactions and plant responses to abiotic stressors (Monsanto, 2010, Tables VIII-3, VIII-5, VIII-6, and VIII-7 and Appendix F, G, H, and I). The only significant difference between MON 87701 and the non-transformed soybean was found between the percentage of hard viable seed at 20ºC (Monsanto, 2010, Table VIII-3). The observed variation in germination was small (0.0% for MON 87701 and 0.5% for A5547), not found under any other temperature regime that ranged between 10 to 30 ºC (Monsanto, 2010 Page 99).

A measure of the reproductive capacity of plants that are propagated by seed, such as soybeans, is the number of seeds that are produced and the germination and viability of those seeds. Overall, MON 87701 produced similar or slightly higher (not significant) percentages of viable seed when compared to A5547 or the null isoline (Monsanto, 2010, Table VIII-3). Interestingly, the tests conducted in Mississippi had a consistent and significant lower germination than the other locations (Monsanto, 2010 Table F-2).

These results on growth characteristics and seed production and germination, indicate that the MON 87701 is not significantly different than its comparators. There is no indication that MON 87701 possesses a selective advantage that would result in increased weediness. Therefore, MON 87701 lacks the ability to persist as a troublesome weed, and there would be no direct impact on current weed management practices for soybean cultivation.

Potential for Gene Flow and Gene Introgression from MON 87701 into its Sexually-Compatible Relatives

Glycine max is cultivated as an annual crop in the U.S. in 43 states(USDA NASS (National Agricultural Statistics Service), 2007). *Glycine max* is generally self-pollinating, (Caviness, 1966; Free, 1970; Monsanto, 2010; Ray et al., 2003). In assessing the risk of gene introgression from MON 87701 to its sexually compatible relatives, APHIS considered two primary issues: 1) the potential for gene flow and introgression, and 2) the potential impact of introgression. Due to the fact that *G. max* lacks wild and cultivated plants with which freely interbreeds, the USDA has determined that any adverse consequences of gene flow from MON 87701 to wild or weedy species in the U.S. are highly unlikely.

Potential for Transfer of Genetic Information to Organisms with which MON 87701 Cannot Interbreed

APHIS assessed whether horizontal gene transfer might occur between MON 87701 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 et al., 2002; Kaneko et al., 2000; 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 (Brown, 2003; Koonin et al., 2001). Third, transgene DNA promoters and coding sequences are optimized for plant expression, not bacterial expression. Thus, even if horizontal gene transfer occurred, proteins corresponding to the transgenes are not likely to be produced. Therefore, APHIS concludes that horizontal gene transfer is unlikely to occur and thus poses no significant environmental or plant pest risk.

Potential for MON 87701 to have Altered Disease and Pest Susceptibilities

APHIS assessed whether MON 87701 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 87701 field trials.

The applicant on Table G-6 (Monsanto, 2010) provided a summary of disease susceptibility evaluated in 131 occasions in 11 different sites, finding no differences between MON 87701 and the control line. Data submitted by Monsanto indicate that there are no significant differences for disease susceptibility between MON 87701 and its comparators (Monsanto, 2010 Field trials summary, Table G-6). However, seed diseases caused by *Cercospora kikuchii* and *Phomopsis* spp. were observed in seeds produced in Mississippi and North Carolina trials (Monsanto, 2010, pages 96 and 104).

Susceptibility to arthropods was also evaluated in 11 sites in 133 occasions finding no differences in pest susceptibility of 20 taxa, except for four species, three of them in the order Lepidoptera (*Plathypena scabra, Pseudoplusia includes* and *Anticarsia gemmatalis*), which is commonly affected by Cry1A proteins, and one species that should not be affected by Cry1 toxins (*Cerotoma trifurcate,* Coleoptera: Chrysomelidae) (Monsanto, 2010, page 295).

MON 87701 was grown during 2007 in 17 locations encompassing the range of geographical and environmental conditions (Monsanto, 2010) where it is anticipated that it will be grown for the intended purpose in the U.S. These field trials included two soybean comparators: MON 87701 and A5547, the comparator. MON 87701 plants were examined for changes in the types of plant pests that were observed and the relative pest damage that those plants incurred (field test summaries submitted for MON 87701 (Monsanto, 2010)) and published information (McPherson et al., 2001; McPherson and MacRae, 2009; Miklos et al., 2007; Walker et al., 2000). When expressed in MON 87701, the Cry1Ac protein provides greater protection from the feeding damage incurred by several important lepidopteran pests of soybeans.

Soybean is not a plant pest in the United States[5], and the introduced DNA in MON 87701 is unlikely to pose a plant pest risk. Based on the analysis of genetic modifications and their functions and field testing data submitted by petitioner, APHIS concludes that there are no significant differences between MON 87701 soybeans and the non-transgenic counterparts relative to pest and disease susceptibility.

Potential Impacts of MON 87701 on Target and Non-target Organisms, Including Beneficial Organisms

For decades, microbial products containing *Bacillus thuringiensis* (the organism that produces the Cry1A protein) have been used to control insect pests on a commercial scale and for home garden applications (Glare and O'Callaghan, 2000; OMRI, 2009; Shelton et al., 2002). Plants that are genetically engineered to express the Cry proteins have a history of safe use in the U.S. Since the mid-1990s, corn and cotton lines have been commercialized without substantiated reports of significant deleterious impacts on non-target organisms (Mendelsohn et al., 2003; OECD, 2007; US-EPA, 2008). The use of transgenic crops producing Cry1A proteins has been shown to reduce the use broad spectrum insecticides[6] without significant impacts on diversity of non-target insects (Cattaneo et al., 2006; Dively, 2005; Marvier, 2007; Naranjo, 2005; Romeis et al., 2006; Torres and Ruberson, 2005; Torres and Ruberson, 2007; Whitehouse et al., 2005). MON 87701 is expected to be similar with respect to the low potential harm to the environment (Monsanto, 2010). Because Cry receptors are not present in non-target birds and mammals (Glare and O'Callaghan, 2000; Hofmann et al., 1988a; Hofmann et al., 1988b; Shimada et al., 2006a; Shimada et al., 2006b; Van Rie et al., 1989; Van Rie et al., 1990), these insecticidal proteins are not expected to adversely affect non-target invertebrate and vertebrate organisms (Glare and O'Callaghan, 2000; US-EPA, 2008).

In addition to the 21 references that the petitioner provided (Monsanto, 2010), other information has been reviewed by APHIS (Carrière et al., 2009; Chen et al., 2009; de Vaufleury et al., 2007; Ferry et al., 2007; Ferry et al., 2006; Ludy and Lang, 2006; Obrist et al., 2006; Rauschen et al., 2009; Schoenly et al., 2003; Sharma and Pampapathy, 2006; Torres and Ruberson, 2008; Vercesi et al., 2006; Zeilinger et al., 2010; Zwahlen and Andow, 2005) that describes the non-effects of Bt crops or toxins in non-target

[5] http://www.aphis.usda.gov/plant_health/plant_pest_info/weeds/downloads/weedlist2006.pdf

[6] Broad spectrum insecticides are chemical insecticides which kill insects that are causing injury to plants and also kill other insects that are not causing injury to the plant. Insects that are inadvertently killed by the application of insecticide are called “non-target” insects. Because Cry proteins are specific for a narrow range of insects, use of Cry1Ac to control plant pests is recognized as being beneficial to the survival of non-target insects US-EPA. (2008) Biopesticides Registration Action Document Bacillus thuringiensis modified Cry1Ab (SYN-IR67B-1) and Vip3Aa19 (SYN-IR102-7) insecticidal proteins and the genetic material necessary for their production in COT102 XCOT67B cotton, U.S. Environmental Protection Agency, Office of Pesticide Programs, Biopesticides and Pollution Prevention Division, Washington, D.C. pp. 135..

organisms, except minor and unclear results found by Zwahlen et al.(2007) using Bt corn (Cry1Ab) as a model.

Based on the above information, APHIS believes that a determination of nonregulated status for MON 87701 will have no adverse effects on non-target organisms. Another consideration by APHIS was the potential that additional 15,000 acres of Cry1Ac-expressing crops may have on the development of Bt resistance in important lepidopteran pests.

Conclusion

APHIS has prepared this plant pest risk assessment in order to determine if event MON 87701 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 87701 is unlikely to pose a plant pest risk.

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