

Monsanto Petition (19-091-01p) for Determination of Non-regulated Status of MON 88702 Cotton

**OECD Unique Identifier:
MON-88702-4**

Draft Plant Pest Risk Assessment

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A. Introduction

Monsanto Company (hereafter referred to as Monsanto) has petitioned the Animal and Plant Health Inspection Service (APHIS) of the United States Department Agriculture (USDA) for a determination that the genetically engineered (GE) insect-protected cotton event MON-88702-4 (hereafter referred to as MON 88702) is unlikely to pose a plant pest risk and, therefore, should no longer be a regulated article under the APHIS' 7 Code of Federal Regulations (CFR) part 340. This petition was assigned the number 19-091-01p, and is hereafter referenced as MON 2019. APHIS administers 7 CFR part 340 under the authority of the plant pest provisions of the Plant Protection Act (PPA) of 2000 (7 U.S.C. 7701 *et seq.*)¹. This plant pest risk assessment was conducted to determine if MON 88702 cotton is unlikely to pose a plant pest risk.

APHIS regulations in 7 CFR part 340 regulate the introduction (importation, interstate movement, or release into the environment) of certain GE organisms and products. A GE organism is no longer subject to the plant pest provisions of the PPA or to the regulatory requirements of Part 340 when APHIS determines that it is unlikely to pose a plant pest risk. A GE organism is considered a regulated article under Part 340 if the donor organism, recipient organism, or vector, or vector agent used in engineering the organism belongs to any genera or taxa designated in 7 CFR 340.2 and meets the definition of plant pest, or is an unclassified organism and/or an organism whose classification is unknown, or any product which contains such an organism, or any other organism or product altered or produced through genetic engineering which the Administrator determines is a plant pest or has reason to believe is a plant pest². MON 88702 cotton was produced by *Agrobacterium*-mediated transformation of cotton (Monsanto 2019), and portions of the introduced genetic sequence come from plant pest organisms listed in 7 CFR 340.2 such as T-DNA border sequences from *Agrobacterium tumefaciens*, an enhancer from figwort mosaic virus, and 3'UTR from cauliflower mosaic virus (Table III-1, pp. 32-34 in Monsanto 2019). Therefore, MON 88702 is considered a regulated article under APHIS regulations at 7 CFR part 340. Monsanto has conducted introductions of MON 88702 as a regulated article under APHIS-authorized notifications since 2011 (Table A-1 of Appendix A, p. 211 in Monsanto 2019), in part, to gather information to support that MON 88702 is unlikely to pose a plant pest risk.

Potential impacts in this Plant Pest Risk Assessment are those that pertain to plant pest risk associated with MON 88702 and its progeny and their use in the absence of confinement relative to the unmodified recipient and/or other appropriate comparators. APHIS utilizes data and information submitted by the applicant, in addition to current literature, to determine if MON 88702 is unlikely to pose a plant pest risk. APHIS

¹Plant Protection Act in 7 U.S.C. 7702 § 403(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."

² Limited exclusions or exemptions apply for certain engineered microorganisms and for interstate movement of some organisms, as in 7 CFR 340.1 and 340.2.(b).

regulations in 7 CFR 340.6(c) specify the information needed for consideration in a petition for nonregulated status. APHIS will assess information submitted by the applicant about MON 88702 related to plant pest risk characteristics; expression of the gene product, new enzymes, or changes to plant metabolism; disease and pest susceptibilities and indirect plant pest effects on other agricultural products; effects of the regulated article on nontarget organisms; weediness of the regulated article; impact on the weediness of any other plant with which it can interbreed; changes to agricultural or cultivation practices that may impact diseases and pests of plants; and transfer of genetic information to organisms with which it cannot interbreed.

APHIS may also consider information relevant to reviews conducted by other agencies that are part of the ‘Coordinated Framework for the Regulation of Biotechnology’ (51 FR 23302 1986; 57 FR 22984 1992). Under the Coordinated Framework, the oversight of biotechnology-derived plants rests with APHIS, the Food and Drug Administration (FDA), and the Office of Pesticide Programs of the U.S. Environmental Protection Agency (EPA). Depending on its characteristics, certain biotechnology-derived products are subjected to review by one or more of these agencies.

EPA regulates under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) (7 U.S.C. 136 *et seq*) the distribution, sale, use and testing of pesticidal substances produced in plants and microbes, including those pesticides that are produced by an organism through techniques of modern biotechnology. EPA also sets tolerance limits for residues of pesticides on and in food and animal feed, or establishes an exemption from the requirement for a tolerance, under the Federal Food, Drug and Cosmetic Act (FFDCA) (21 U.S.C. Chapter 9). Prior to registration for a new use for a new or previously registered pesticide, EPA must determine through testing that the pesticide does not cause unreasonable adverse effects on humans, the environment, and non-target species when used in accordance with label instructions. EPA must also approve the language used on the pesticide label in accordance with 40 CFR part 158. Other applicable EPA regulations include 40 CFR part 152 - Pesticide Registration and Classification Procedures, part 174 - Procedures and Requirements for Plant Incorporated Protectants (PIPs) and part 172 - Experimental Use Permits. EPA has granted a permanent exemption for residues of mCry51Aa2 in food and feed (*Bacillus thuringiensis* Cry51Aa2.834_16; Exemption From the Requirement of a Tolerance; 83 FR 3601; January 26, 2018)(US-EPA 2018b). EPA has also granted a time-limited registration for MON 88702 cotton under FIFRA section 3(c)(5) for purposes of agronomic evaluation, seed increase, and production in breeding nurseries, both as a single event and as a stack with other insecticidal events (US-EPA 2018a, 2019).

The FDA under the FFDCA is responsible for ensuring the safety and proper labeling of all plant-derived foods and feeds, including those developed through modern biotechnology. To help sponsors of foods and feeds derived from genetically engineered crops comply with their obligations, the FDA encourages them to participate in its voluntary early food safety evaluation for new non-pesticidal proteins produced by new plant varieties intended to be used as food (US-FDA 2006) and a more comprehensive voluntary consultation process prior to commercial distribution of food or feed (57 FR

22984 1992). Monsanto has completed the consultation process with the FDA for MON 88702 (US-FDA 2018).

B. Development of MON 88702 Cotton

Cotton belongs to the genus *Gossypium*, which consists of approximately 50 species, four of which are generally cultivated in tropical and subtropical regions around the world (Fryxell 1984; Percival et al. 1999; OECD 2008). Upland cotton, *G. hirsutum*, is the predominant cultivated species in the United States, at approximately 98% of the cotton crop, while Pima cotton, *G. barbadense* is grown in smaller amounts (USDA-NASS 2019b). There are two other cultivated species, *G. arboreum* and *G. herbaceum*, but they are not grown in the U.S. or its territories. Cotton is a perennial plant cultivated as an annual, and is grown in 17 states from Virginia southward and westward to California - an area often referred to as the Cotton Belt (Figure 1ab from (USDA-NASS 2019a, c)).

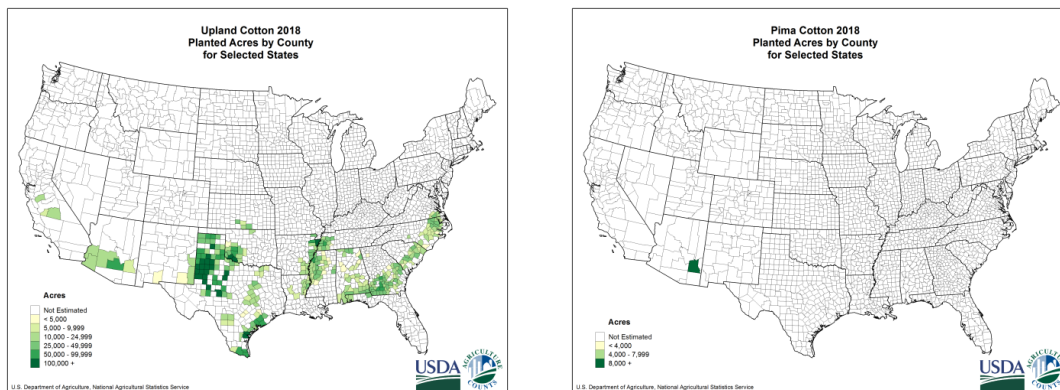


Figure 1: Planted acres of cotton by county in the US in 2018 for **a)** Upland and **b)** Pima varieties (USDA-NASS 2019a, c)

Historically, boll weevil (Coleoptera: Curculionidae, *Anthonomus grandis*) and boll-feeding Lepidoptera have been the primary insect pests damaging cotton. In fact, USDA-APHIS has led two successful eradication programs for these pests: one for boll weevil, and one for pink bollworm (Lepidoptera: Gelechiidae, *Pectinophora gossypiella*). For control of pink bollworm, as well as other boll-feeding Lepidoptera, such as bollworm (*Helicoverpa zea*) and tobacco budworm (*Heliothis virescens*), varieties of Bt cotton have been developed and have been in cultivation since the late 1990s. Bt cotton targeting Lepidoptera pests is now prevalent in cotton-growing regions, planted on 92% of cotton acres in the US (USDA-NASS 2019b).

However, due to increased Bt cotton cultivation for Lepidoptera pests and the success of the above-mentioned eradication campaigns, pests that were previously controlled by widescale use of insecticides for these primary pests have risen in importance over the last 30 years, including pests in Hemiptera and Thysanoptera (Naranjo 2011; Luttrell et al. 2015). The tarnished plant bug *Lygus lineolaris*, Miridae and the Western tarnished plant bug *Lygus hesperus* are both in the family Miridae within the order Hemiptera.

Lygus lineolaris is prevalent in the mid-South, and *L. hesperus* is more prevalent in western cotton-growing regions. When a species designation of tarnished plant bug is not necessary in this document, “Lygus” may be used for simplicity. While Lygus primarily causes damage to reproductive structures on the cotton plant, thrips (Thysanoptera: Thripidae) are primarily problematic at the seedling stage. Because seedlings are especially sensitive to early-season damage, thrips control is done primarily by insecticidal seed treatments and/or in-furrow sprays at planting (Cook et al. 2011; Allen et al. 2018; North et al. 2018).

MON 88702 was developed to provide protection to cotton plants from Lygus and thrips. It is the first Bt crop variety to target this group of insect pests and to produce a member of this novel class of cry proteins derived from *Bacillus thuringiensis*. Bt crops in general, however, are not novel to the environment. Bt cotton and Bt corn are widely cultivated, with Bt cotton targeting Lepidoptera pests and Bt corn targeting Lepidoptera and/or Coleoptera pests. EPA-registered plant-incorporated protectants (defined above) in cotton include multiple 3-domain crystalline (Cry) toxins, and one VIP (vegetative insecticidal protein) toxin, while EPA-registered PIPs in corn include 3-domain and binary cry toxins as well as a VIP protein. Commercial registrations for these products can be found on the EPA website: <https://www.epa.gov/ingredients-used-pesticide-products/current-and-previously-registered-section-3-plant-incorporated>.

C. Description of Inserted Genetic Material, Its Inheritance and Expression, Gene Products, and Changes to Plant Metabolism

To inform the potential hazards resulting from the genetic modification and potential routes of exposure related to the inserted DNA and its expression products, APHIS assessed data and information presented in the petition related to: the transformation process; the source of the inserted genetic material and its function in both the donor organism and the GE crop event; and the integrity, stability and mode of inheritance of the inserted genetic material through sexual or asexual reproduction based on the location of the insertion (e.g. nucleus or organelle) and the number of loci inserted.

APHIS also assessed data presented in the petition on whether the genetic modification results in expression of new genes, proteins, or enzymes or changes in plant metabolism or composition in the MON 88702 relative to DP393 (its nontransgenic counterpart) and other reference cotton lines. The assessment encompasses a consideration of the expressed protein and any observed or anticipated effects on plant metabolism including, e.g. any relevant changes in levels of metabolites, nutrients, or antinutrients in cottonseed derived from the GE crop event compared to those in the conventional reference counterparts.

This information is used later in this risk assessment to inform whether there is any potential for plant pest vectors or sequences to cause disease or greater plant pest risks in the GE crop event; or for expression of inserted DNA, new proteins or enzymes, or changes in metabolism to affect plant pest or diseases, nontarget beneficial organisms,

weediness, agricultural practices that impact pest or diseases or their management, or plant pest risks through horizontal gene flow.

Description of the genetic modification and inheritance of inserted DNA

MON 88702 cotton was produced by disarmed *Agrobacterium*-mediated transformation of conventional cotton variety DP393 with a plasmid designated PV-GHIR508523. The plasmid vector contains two sections of T-DNA (Figure 2): one containing the mCry51Aa2 cassette (T-DNA I), and one containing a cassette with the *aadA* (aminoglycoside 3'-adenyl transferase) selectable marker conferring spectinomycin and streptomycin resistance (T-DNA II). After successful transformation, plant lines were subsequently segregated and screened for those that only contained the mCry51Aa2 cassette (see table below). Transformants were cleared of disarmed *Agrobacterium* by use of antibiotics.

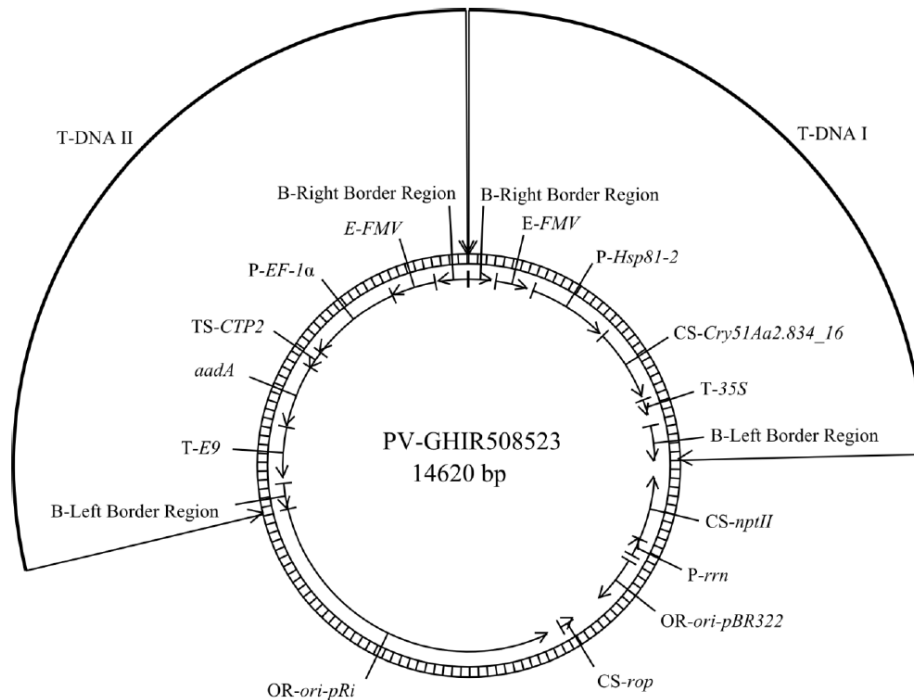


Figure 2: Plasmid map of PVGHIR508523 used to develop MON 88702 cotton (from Monsanto, 2019).

In summary, MON88702 contains the mCry51Aa2 coding sequence that is driven by a heat shock promoter from *Arabidopsis thaliana* and enhancer sequence from figwort mosaic virus. Transcription termination is controlled by the 35S 3'UTR from cauliflower mosaic virus. None of the inserted sequences from plant pests encode a plant pest or infectious agent. Copied below is the information provided by the petitioner (Table IV-1, p. 44 in Monsanto 2019).

Summary of Genetic Elements in MON 88702

Genetic Element	Location in Sequence	Function (Reference)
Flanking DNA	1-1642	Flanking DNA
B1-Right Border Region <i>rI</i>	1643-1710	DNA region from <i>Agrobacterium tumefaciens</i> containing the right border sequence used for transfer of the T-DNA (Depicker et al. 1982; Zambryski et al. 1982).
Intervening Sequence	1711-1763	Sequence used in DNA cloning.
E2-FMV	1764-2170	Enhancer from the 35S RNA of figwort mosaic virus (FMV) (Richins et al. 1987) that enhances transcription in most plant cells (Rogers 2000).
Intervening Sequence	2171-2245	Sequence used in DNA cloning
P3-Hsp81-2	2246-3253	Promoter and 5' UTR leader sequence for the heat shock protein 81-2 (Hsp81-2) from <i>Arabidopsis thaliana</i> that directs transcription in plant cells.
Intervening Sequence	3254-3290	Sequence used in DNA cloning.
CS4-Cry51Aa2.834_16	3291-4211	Coding sequence of the modified Cry51Aa2 protein of <i>Bacillus thuringiensis</i> that provides insect resistance (Baum et al. 2012; Anderson et al. 2015; !!! INVALID CITATION !!!).
Intervening Sequence	4212-4243	Sequence used in DNA cloning.
T5-35S	4244-4443	3' UTR sequence of the 35S RNA of cauliflower mosaic virus (CaMV) (Mogen et al. 1990) that directs polyadenylation in plant cells.
Intervening Sequence	4444-4581	Sequence used in DNA cloning.
B- Left Border Region <i>rI</i>	4582-4785	DNA region from <i>Agrobacterium tumefaciens</i> containing the left border sequence used for transfer of the T-DNA (Barker et al. 1983).
Flanking DNA	4786-6748	Flanking DNA.

Using an approach that combined next-generation sequencing of the genome and directed sequencing of specific elements, Monsanto confirmed a stable, single insertion of the T-DNA I cassette as described in the table above. No vector backbone or T-DNA II elements are present in MON 88702. The intended T-DNA I cassette was shown to be stably integrated at a single locus and inherited at expected Mendelian rates.

Regarding the gene of interest itself, the mCry51Aa2 protein (mCry51Aa2.834_16) expressed in MON88702 shares approximately 96% sequence similarity from the wild-type protein, with 8 amino acid substitutions and 3 deletions (Gowda et al. 2016). However, USDA notes that the extent of these directed mutations, while necessary for improvement of the introduced protein, actually results in the mCry51Aa2 protein sharing 99% amino acid similarity with the wild-type Cry51Aa1 protein (BLAST search conducted Apr 15 2019, NCBI). The Cry51Aa1 and Cry51Aa2 toxins have activity against hemipteran and coleopteran insects (Anderson et al. 2015; Baum et al. 2015; Xu et al. 2015). While it is possible that this protein could be classified as a Cry51Aa1 (Crickmore et al. 1998), the designation mCry51Aa2 will be used for simplicity and precedent.

Expression of inserted DNA, changes in gene expression, new proteins or metabolism

Expression of mCry51Aa2 is under the control of a heat shock promoter from *Arabidopsis* and an enhancer sequence derived from figwort mosaic virus, resulting in constitutive expression in the plant. Though constitutive, however, expression is not necessarily static across time and tissues. The highest protein amounts were observed in leaves and squares, while intermediate amounts were quantified in roots and seeds. Protein amounts were generally low in pollen.

Expression of the mCry51Aa2 protein in MON 88702 results in protection of the plant from *Lygus* and thrips feeding. Efficacy data and mortality of target pest(s) are provided in the petition. Potential impacts to nontarget organisms and the relationship to protein expression are addressed in the respective section below.

Other than the introduced mCry51Aa2 protein, no other changes in plant proteins or metabolism are reported or anticipated. Compositional analyses were done on MON 88702 seed to determine if there were any relevant changes compared to those in the parental line and additional conventional lines as a reference. No significant differences were found in thirty metabolites, nutrients, and antinutrients compared to control and reference cotton lines.

In summary, the molecular characterization, protein expression, and compositional analysis of MON 88702 cotton support a conclusion that there are no unanticipated changes in the GE plant line other than the intended expression of the mCry51Aa2 introduced protein.

D. Potential Plant Pest and Disease Impacts

APHIS assessed whether potential plant pest or disease impacts are likely to result from the transformation process, from DNA sequences from plant pests, or from any other expression products, new enzymes, proteins or changes in plant metabolism or composition in MON 88702 that are known or anticipated to cause disease symptoms, or to affect plant pests or diseases or plant defense responses (as identified from the previous section). APHIS also assessed whether MON 88702 is likely to have significantly increased disease and pest susceptibility based on data and observations from field trials on specific pest and disease damage or incidence and any agronomic data that might relate to such damage. Impacts or changes were assessed to determine if they would (1) affect the new GE crop and/or result in significant introduction or spread of a damaging pest or disease to other plants; (2) result in the introduction, spread, and/or creation of a new disease; and/or (3) result in a significant exacerbation of a pest or disease for which APHIS has a control program. Any increase in pest or disease susceptibility is evaluated with respect to the context of currently cultivated varieties, the ability to manage the pest or disease, and the potential impact on agriculture.

Plant Protection and Quarantine (PPQ) is an APHIS program that safeguards agriculture and natural resources from the entry, establishment, and spread of animal and plant pests

and noxious weeds into the United States of America; and supports trade and exports of U.S. agricultural products. PPQ responds to many new introductions of plant pests to eradicate, suppress, or contain them through various programs in cooperation with state departments of agriculture and other government agencies. These may be emergency or longer term domestic programs that target a specific pest. A variety of insect, plant disease, mollusk, nematode or weed programs exist (USDA-APHIS 2020). PPQ has eradication programs for two cotton pests, boll weevil and pink bollworm, but these insects are not targeted by the insecticidal protein in MON 88702.

Monsanto conducted field studies to assess the phenotypic, agronomic, and environmental interaction characteristics of MON 88702. In a combined-site analysis, there were no significant differences between MON 88702 and the conventional control in susceptibility to any diseases detected in the field season that could infect cotton. Likewise, there were no biologically meaningful differences observed in any agronomic studies or compositional analyses. Regarding arthropod damage, there were no significant differences in susceptibility to a wide variety of cotton pests. Two exceptions were slightly decreased damage to MON 88702 from bollworms and stink bugs, respectively. The difference in bollworm damage was only found at one site out of 8 locations in one season, and it was a difference between “none” and “slight”. Especially given the lack of toxicity of mCry51Aa2 to Lepidoptera in lab assays, this difference is not regarded as biologically significant. When examining the differences in stink bug damage, this effect was not consistent across sites or years. Since mCry51Aa2 was developed for purposes of controlling thrips and Hemiptera, some reduced damage in MON 88702 due to stink bugs (Hemiptera: Pentatomidae) may not be unexpected, even if not at commercially acceptable or consistent levels of control.

Other than the intended effect on the target pests, the introduced genes did not significantly alter the observed insect pest infestation and disease occurrence or resulting damage on MON 88702 over the control line. As discussed above, there were no significant changes in MON 88702 composition that would render MON 88702 more susceptible to pests and diseases over its control or reference cotton varieties. The observed agronomic traits also did not reveal any significant changes that would indirectly indicate that MON 88702 is or could be relatively more susceptible to pests and diseases over control or reference cotton varieties. Thus MON 88702 is unlikely to be more susceptible to plant pathogens and insect pests than conventional cotton. For this reason, MON 88702 is unlikely to differ from conventional cotton in its ability to harbor or transmit plant pathogens or pests and cause indirect plant pest effects on other agricultural products.

E. Potential Impacts on Nontarget Organisms Beneficial to Agriculture

The GE plant is engineered for pest resistance. APHIS assessed whether exposure or consumption of the GE plant and the PIP would have a direct or indirect adverse impact on species beneficial to agriculture. Organisms considered were representatives or surrogates of the species associated with production of the regulated crop in the

agricultural environment. The assessment includes an analysis of toxicity and specificity of the PIP and exposure to sensitive nontarget organisms in the agricultural environment of the GE plants. It also may include an analysis of the GE plant compared to the non-GE counterpart (or other comparators) with respect the following: any biologically relevant changes in the phenotype or substances produced which may be novel or expressed at significantly altered amounts that are associated with impacts on organisms beneficial to agriculture, and/or any observations of beneficial organisms associated with the plants.

The modified mCry51Aa2 protein (Cry51Aa2.834_16) expressed in MON 88702 shares approximately 96% sequence similarity from the wild-type protein, with 8 amino acid substitutions and 3 deletions (Gowda et al. 2016). However, USDA notes that the extent of these directed mutations, while necessary for improvement of the introduced protein, actually results in the mCry51Aa2 protein sharing 99% amino acid similarity with the wild-type Cry51Aa1 protein (BLAST search conducted Apr 15 2019, NCBI). The Cry51Aa1 and Cry51Aa2 toxins have activity against hemipteran and coleopteran insects (Anderson et al. 2015; Baum et al. 2015; Xu et al. 2015). While it is possible that this protein could be classified as a Cry51Aa1 (Crickmore et al. 1998), the designation mCry51Aa2 will be used for simplicity and precedent.

Regarding protein structure, the mCry51Aa2 protein is classified within ETX/MTX2 toxin group, referring to domains found in epsilon and mosquitocidal toxins with beta protein sheets. Epsilon toxin is toxic against vertebrates; however, even though there is some domain similarity between epsilon toxin and mCry51Aa2, it is important to note that there is no amino acid similarity in regions likely conferring specific toxicity in insects (Moar et al. 2017), and, furthermore, that no toxicity was observed to vertebrates in tests for acute toxicity using bobwhite quail and mouse (Section V.B.5.2 in (Monsanto 2019)). The mCry51Aa2 protein forms an inactive dimer in solution, but when treated with trypsin, is able to bind to the midgut in *Lygus* (Jerga et al. 2016).

The mCry51Aa2 protein was tested for toxicity with a range of invertebrate species, especially insects. Species were selected based on amenability to lab testing, to encompass a variety of species across the taxonomic spectrum, and to include species representing different ecological niches. (Further testing was conducted on species related to the target pest, discussed below.) Protein amounts used in bioassays were either similar to or greater than what was quantified in the plant, in order to support a conclusion that these groups would not be negatively impacted by protein exposure expected in the environment. Mortality to target pests, such as *Lygus* spp. and thrips, was evidenced through diet bioassays and/or efficacy tests on MON 88702 plants. No toxicity was observed in earthworms, Collembola, Hymenoptera, Lepidoptera, or Neuroptera. Furthermore, no toxicity was observed in tests with *Aedes* mosquito larvae (Monsanto 2019), so effects on non-target Diptera are also unlikely.

Regarding Coleoptera, no mortality was observed in three species of predatory beetles representing two families: Coccinellidae and Staphylinidae (Monsanto 2019). For beetles within Chrysomelidae (also known as leaf beetles, which are herbivores), one species exhibited no mortality (western corn rootworm, *Diabrotica virgifera virgifera*), and two

species exhibited some mortality. For Colorado potato beetle (*Leptinotarsa decemlineata*) and southern corn rootworm (*Diabrotica undecimpunctata howardi*), some mortality was observed, but in both cases 100% mortality was not reached with increasing concentrations. Mortality curves for both species leveled off around 40-50%. Because high levels of mortality were never reached for any beetles that were tested, any negative effect that might be possible is expected to be minor.

There are numerous nontarget species beneficial to agriculture, which are related to the target pests. *Lygus* spp. are common pests of cotton and other crop plants, as are other members of the family Miridae (plant bugs), but there are also predaceous species in this family (Wheeler 2000), and broadly in the suborder Heteroptera. Predatory Heteroptera are common in cotton (Head et al. 2005; Naranjo 2005; Torres and Ruberson 2005) and contribute significantly to natural pest suppression in agroecosystems (Schaefer and Panizzi 2000). Thrips (Thysanoptera) are also listed as target pests of MON 88702 and are important pests in seedling cotton. Most thrips species tend to be herbivorous, and obligate predators are not common in Thysanoptera (Mound 2005), but some examples exist (Coville and Allen 1977). However, predator thrips were not tested due to difficulty in developing an assay and relatively lower importance as natural enemies in cotton, compared to predatory Heteroptera.

Emphasis was placed on predatory Heteroptera because of their relatedness to the target pests and importance as natural enemies in agroecosystems. Furthermore, many predatory Heteroptera are known to engage in herbivory in order to supplement a primarily prey-based diet (Naranjo and Gibson 1996). These predators are known to feed on numerous plant species (Crocker and Whitcomb 1980; Wiedenmann et al. 1996; Eubanks and Denno 1999), including cotton (Ridgway and Jones 1986; Tillman and Mullinix 2003). Some examples of Heteroptera commonly found in agroecosystems, and cotton in particular, include *Orius* (Anthocoridae), *Nabis* (Nabidae), *Geocoris* (Geocoridae), *Zelus* (Reduviidae), and *Podisus* (Pentatomidae). The developer and/or a collaborator conducted bioassays on members of some of these taxa, including *Orius insidiosus*, *O. majusculus*, *Nabis alternatus*, *Geocoris punctipes*, and *Zelus renardii* (Monsanto 2019).

Orius spp. and other Anthocorids (minute pirate bugs) are important predators in many environments, including cotton agroecosystems. The tests with *Orius* conducted by the developer or a collaborator are summarized below:

Using recombinant protein, equivalent to that produced in MON 88702, incorporated into an artificial diet system, *Orius insidiosus* (insidious flower bug) 5-day-old nymphs were tested with a range of concentrations of mCry51Aa2 in order to obtain a survivorship curve. However, mortality leveled off at around a concentration of 100 ug/ml of protein in artificial diet, at which point mortality did not exceed 50% with increasing concentrations of protein (Monsanto 2019). No sublethal effects or developmental delays were observed. The plateau effect in mortality with increasing concentration of mCry51Aa2 in artificial diet lends some uncertainty to conclusions of no likely impact of MON 88702 to nontarget organisms.

Further tests were conducted using MON 88702 plant material as a basis, in order to get a more detailed understanding of any potential effects to *Orius* spp. Tests were done with plant material only (because *Orius* is known to engage in limited herbivory), and plant material in combination with two-spotted spider mites (TSSM; *Tetranychus urticae*) as prey. TSSM were selected as prey items in trophic-level testing because they are common pest herbivores in cotton and therefore common prey for predators, and also because they have been reported to contain relatively higher amounts of plant-incorporated Cry proteins than other herbivorous arthropods feeding on these plants (Dutton et al. 2002; Obrist et al. 2006). The reason or mechanism by which TSSM may accumulate or sequester plant-incorporated Cry proteins, sometimes even at levels higher than what is measured in the plant itself (Torres and Ruberson 2008), is unknown.

Five-day-old nymphs of *O. majusculus* that were provided with *ad libitum* leaf material and spider mites that had fed on leaf material indicated no mortality on MON 88702; some sublethal effects were, however, observed. Additional tests were conducted with one-day-old nymphs of *Orius*, because for many toxins, younger stages tend to be more susceptible. Using one-day-old nymphs of *O. majusculus* and *O. insidiosus*, significant mortality was observed when provided with leaf material of MON 88702 and TSSM as prey. When one-day-old nymphs of *O. insidiosus* were provided with TSSM and eggs of *Ephestia kuehniella* (Lepidoptera), no significant difference in mortality was observed between MON 88702 and the control. However, this test confounds survival with behavior (choice), and extrapolating results to the field would be highly dependent on whatever potential prey items are available at any given time.

Regarding other predatory Heteroptera, bioassays with recombinant mCry51Aa2 incorporated into artificial diet were developed. Two concentrations (400 and 4000 ug/g protein in diet; for comparison, see below for protein amounts quantified in plants) were tested on *Geocoris punctipes*, *Nabis alternatus*, and *Zelus renardii*. No significant differences in mortality were observed for these species at either concentration. Sublethal effects were, however, observed. For all three species, there were developmental delays and reduced body mass at the 4000 ug/g concentration. At the 400 ug/g concentration, smaller developmental delays were observed in all three species; reduced body mass was additionally observed in *Z. renardii*.

Assessment of risk to nontarget organisms is informed by expression in the plant and exposure pathways. Expression in mCry51Aa2 in MON 88702 was reported from different tissues in cotton plants collected from multiple sites in two field seasons. Though different methods of collection and quantification were used in the two years of data and furthermore, variability in expression is to be expected, some trends can be observed. Protein expression was highest overall in squares, with a 95th percentile concentration of approximately 770 ug/g fresh weight, followed by leaves with a 95th percentile concentration just below 500 ug/g. This result would be desirable, given that the target pests feed on the aboveground portion of the plant. Roots and seeds had lower expression at approximately one level of magnitude, and protein concentration in pollen was lower still at around 2-5 ug/g. (Note that mCry51Aa2 quantification in MON 88702

as originally reported in Gowda et al. (2016) would indicate significantly higher expression, but the authors have corrected the typographical error (Gowda et al. 2020).)

Exposure of nontarget organisms to mCry51Aa2 in MON 88702 can occur directly or indirectly. Direct exposure could occur, for example, by a pollinator feeding on pollen and nectar, a scavenger or detritivore feeding on directly on the plant or sloughed-off material, parasitoids feeding on nectar, or predators feeding on pollen or vegetative plant parts. Natural enemies that feed on plants may do so to obtain moisture, to sustain themselves in periods of prey scarcity, or to supplement their diet when sufficient prey are present (Lundgren 2009). Predator Heteroptera in particular, are known to feed on green plant tissues and to derive nutritional benefit supplemental to a diet with optimal prey (eg. Kiman and Yeargan 1985; Ruberson et al. 1986; Gillespie and McGregor 2000). Indirect exposure is generally understood to constitute trophic exposure, whereby a natural enemy is exposed to the plant-produced toxin via a prey item. Use of prey species that are not susceptible to the toxin excludes any confounding effects of intoxicated or suboptimal prey. Finally, any community-level effects due to a plant-incorporated toxin would not be a direct result of exposure to any toxin, but could be considered an indirect effect of a plant-produced toxin through removal of some target species, for example (Gonzalez and Wilson 1982).

The developer conducted extensive field sampling for arthropods in the cotton community to ascertain whether MON 88702 could have impacts to nontarget organisms in a real-world scenario. Sampling was conducted in three field seasons, at 5-6 sites in various locations in cotton growing regions of the US. Treatments included MON 88702, its untreated parental control line DP 393, and treatments that included broad-spectrum and selective insecticides for comparison. Results were presented both by site and across sites, and analysis was done on transformed count data rather than a generalized linear model.

In the 2018 season, there was more focus on predator Heteroptera. Though not statistically significant, counts of *Geocoris*, *Nabis*, and *Zelus* were always lower in MON 88702 compared to DP393 (Table V-13 and V-14 in Monsanto 2019). Significant differences were observed, however, when comparing unsprayed MON 88702 to DP393 sprayed with broad-spectrum insecticide. No significant differences in abundance of *Orius* in MON 88702 compared to unsprayed DP393 were observed (Tables V-9 and V10 in Monsanto 2019).

Though no significant differences in arthropod abundance were found, a few caveats should be noted. First, a power analysis is provided that calculates the ability to detect a 50% difference, but when reductions in the target pest are approximately 40-60% (Akbar et al. 2019; Monsanto 2019), then perhaps a lower percentage should be used for examining effects to nontarget organisms. Second, sampling date can be a significant contributor to variance in the dataset (eg. Whitehouse et al. 2005; Whitehouse et al. 2007; Reay-Jones et al. 2016). Populations of both pests and natural enemies are expected to exhibit fluctuations, either trending over a season, or simply variable by sampling date. If there were low-level lethal effects or sublethal effects on natural enemies, such an impact

would have a cumulative effect over the growing season, which is not analyzed or depicted.

APHIS notes that these studies are complex and time-consuming and that there are some inherent difficulties with extracting strong conclusions from field abundance data, which is known to be highly variable. Macfayden and Zalucki (2012) discuss some of the issues associated with field studies on arthropod abundance; though their focus is on chemical insecticides, many of the same considerations apply to insecticidal plants. One example is time of day for collecting samples; some arthropods are more active at different times of day, or even nocturnal (Pfannenstiel and Yeargan 2002; Pfannenstiel 2005; Wade et al. 2006).

In summary, impacts to nontarget organisms are expected to be restricted to those related to the target pests. Effects to predator beetles is possible, but not likely. Effects to predator Heteroptera and predator thrips is possible, but a significant negative impact is unlikely, based on bioassay data in the laboratory and data from field sampling.

Therefore, based on the above analysis, APHIS concludes that exposure to and/or consumption of the GE plant and PIP are unlikely to have a significant adverse impact to nontarget organisms beneficial to agriculture.

F. Potential for Enhanced Weediness of MON 88702

APHIS assessed whether the GE crop event is likely to become more weedy (i.e. more prevalent, competitive, damaging or difficult-to-control in situations where it is not wanted) than the nontransgenic progenitor from which it was derived, or other varieties of the crop currently under cultivation. The assessment considers the basic biology of cotton, the situations in which cotton volunteers or feral populations are considered weeds, and an evaluation of the MON 88702 cotton event compared to the nontransgenic counterpart DP393 evaluated under field (and/or lab) conditions characteristic for the regions of the U.S. where the GE crop is intended to be grown. For this crop, such characteristics include germination, dormancy, phenotypic, agronomic, and environmental interaction characteristics of MON 88702 cotton were evaluated in a comparative manner to assess weediness. For all of these characteristics measured, MON 88702 did not exhibit any weediness traits that were outside the reference range (Monsanto 2019).

In the U.S., cotton is not listed as a noxious weed species by the federal government (7 CFR part 360), nor is commercial cotton considered to be a weed (Randall 2017). Further, cotton does not possess weedy characteristics. Cotton is sensitive to low temperature, as temperatures below 15°C or above 38°C will cause growth arrest and freezing temperature even kill cotton plants (OECD 2008; Freeland et al. 2010). The complete growth cycle of cotton generally requires above 15°C for more than 150 days. The low-temperature sensitivity of cotton restricts its geological distribution. Cultivated cotton rarely displays any dormancy characteristics, but it may grow as a volunteer under

favorable conditions (OECD 2008). If any seeds remain viable and germinate the following year, mechanical methods or herbicides can be used to control volunteers.

Based on the agronomic field data, including response to environmental stressors, and literature survey concerning weediness potential of the crop, MON 88702 is unlikely to persist as a troublesome weed or to have an impact on current weed management practices. This conclusion is further supported by the lack of biologically meaningful differences in germination and dormancy characteristics of seed. Finally, extensive post-harvest monitoring of field trial plots planted with the GE crop under USDA-APHIS notifications or permits (Monsanto 2019) did not reveal any differences in survivability or persistence relative to other varieties of the same crop currently being grown. These data suggest that MON 88702 is no more likely to become a weed than conventional varieties of the crop.

G. Potential Impacts on the Weediness of Any Other Plants with which Mon 88702 Cotton Can Interbreed

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; Rieseberg and Wendel 1993; Soltis et al. 1993; 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). It has been a common practice by plant breeders to artificially introgress traits from wild relatives into crop plants to develop new cultivars (Khoury et al. 2013). 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 a few other crops (see Table 1 in Ellstrand et al. (1999)). This topic is covered in two sections: 1) the potential for gene flow, hybridization and introgression from the GE crop event to sexually compatible relatives, including wild, weedy, feral or cultivated species in the United States and its territories, and 2) if so, the risk potential with respect to weediness of those taxa based on the phenotypic changes that have been observed in the engineered plants.

Potential for gene flow, hybridization and gene introgression

The reproductive biology and pollination characteristics of cotton are well known and have previously been described (OECD 2008). Cotton has complete flowers and is predominately self-pollinating. However, cross-pollination can also occur at generally low levels in the presence of suitable insect pollinators (McGregor 1976; Van Deynze et al. 2005b; OECD 2008). Normally, the occurrence of transgene introgression through pollen dispersal depends on several factors such as genetic compatibility, mode of pollination, sympatric distribution, etc. A few prerequisites for successful gene flow are: i) presence of sexually compatible relatives; ii) sympatric distribution of GE and its sexually compatible taxa; and iii) overlapping phenology between GE and its sexual compatible taxa (Pleasants and Wendel 2005). In general, the extent of transgene

introgression will depend on the species pool, preferences, and abundance of pollinators, which can vary according to region, location, season, time of day, and use of insecticides (OECD 2008). In addition, transgene introgression will decrease with increasing geographic distance between the source and receiver populations and physical barriers (McGregor 1976; Umbeck et al. 1991; Van Deynze et al. 2005a; Zhang et al. 2005; OECD 2008). Field studies demonstrate that upland cotton outcrossing rate declines exponentially with distance from the pollen source, typically below 1% beyond 10 meters (Van Deynze et al. 2005a).

As described above in Section B, the genus *Gossypium* consists of 50 species, including four cultivated species, two allotetraploids - *G. hirsutum* and *G. barbadense* ($2n = 4x = 52$) and the two diploids - *G. arboreum* and *G. herbaceum* ($2n = 2x = 26$) (OECD 2008; Pleasants and Wendel 2010; Wendel et al. 2010). In United States and its territories, there exist only two domesticated allotetraploid species, *G. hirsutum* (upland cotton) and *G. barbadense* (Pima or Egyptian cotton), and two wild species, *G. thurberi* and *G. tomentosum* (USDA-NRCS 2020b). The two wild species are native to the United States. *G. thurberi* and *G. tomentosum* grow in Arizona and Hawaii, respectively (USDA-NRCS 2020b). Upland cotton is sexually compatible with the tetraploids *G. barbadense* and *G. tomentosum*, and it can form viable progeny with both species (OECD 2008). Thus, unassisted outcrossing and gene introduction could potentially occur in areas where these species are co-located. However, upland cotton as a tetraploid is effectively incompatible with diploid species such as *G. thurberi*, and they cannot normally hybridize in natural settings and produce fertile offspring (OECD 2008).

Native populations of *G. hirsutum* grow in Florida, Puerto Rico, and the Virgin Islands, while introduced populations grow in some of the Hawaiian Islands (Fryxell 1979; Coile and Garland 2003; Wagner et al. 2012; Lee and Fang 2015; USDA-NRCS 2020a; Wunderlin et al. 2020). However, due to eradication efforts to control pink bollworm, native and feral populations of *G. hirsutum* have become very rare in the major U.S. cotton growing areas, and it has even been listed as endangered by the state of Florida (Coile and Garland 2003; Florida DCAS 2019). In Florida, the naturalized populations of *G. hirsutum* are separated by over 120 miles from the nearest commercial cotton production areas in the Florida panhandle (Calhoun County, FL) (Wunderlin et al. 2020). Additionally, in Puerto Rico and the Virgin Islands, there are no commercial cultivation of cotton. Thus, outcrossing from MON 88702 to wild *G. hirsutum* is highly unlikely.

Gossypium barbadense is cultivated in many areas where *G. hirsutum* is also grown (USDA-NASS 2019b). Naturalized populations of *G. barbadense* grow in Puerto Rico, the Virgin Islands and most of the Hawaiian Islands, but it is no longer widely grown as an agricultural commodity in Hawaii (Wagner et al. 1990; Pleasants and Wendel 2010; USDA-NASS 2019b). Although both *G. barbadense* and *G. hirsutum* are predominantly self-pollinating, cross-pollination via insect pollinators can occur both within and between the species (Brubaker et al. 1993; Van Deynze et al. 2005b; Llewellyn et al. 2007; OECD 2008). It has been reported that there is an asymmetrical gene flow between *G. hirsutum* and *G. barbadense*. For example, relatively little gene introgression were found from *G. hirsutum* (male parent) into native or naturalized *G. barbadense* (female parent) in Central America and the Caribbean (Fryxell 1979), while introgression in the

reverse direction i.e. from *G. barbadense* (male parent) to native or naturalized *G. hirsutum* (female parent) were found to be relatively common (Wendel et al. 1992; Brubaker et al. 1993). This asymmetry in gene flow between native or naturalized *G. hirsutum* and *G. barbadense*, and the lack of commercial cotton production in Hawaii, Puerto Rico and the Virgin Islands suggest that gene introgression from cultivated MON 88702 to native or naturalized *G. barbadense* is highly unlikely.

The above asymmetric gene flow observed between native and naturalized populations, however, is directionally opposite from gene introgression in modern cultivars of *G. hirsutum* and *G. barbadense*. Specifically, in modern cultivars, the introgression from *G. hirsutum* (male parent) into *G. barbadense* (female parent) is common whereas the introgression from *G. barbadense* (male parent) into *G. hirsutum* (female parent) is rare (Brubaker et al. 1993; Van Deynze et al. 2011; Wendel et al. 1992). Nevertheless, introgression of MON 88702 cotton into *G. barbadense* cultivars is expected to be at similar low levels observed between cultivated cotton varieties, which also depends on spatial isolation distance and other factors. It is reported that Upland/Pima hybrid plants have been observed at a rate of 0.01% in fields sown with seeds of cultivated varieties that were obtained from production fields separated by at least 800 meters (Van Deynze et al. 2005a).

The wild cotton tetraploid species *G. tomentosum* is sexually compatible with upland cotton. However, *G. tomentosum* populations are limited to the Hawaiian Islands. Furthermore, there has been no commercial cotton cultivation in Hawaii, and APHIS has no record showing that seed companies use the Hawaiian Islands as a cotton winter nursery. All these facts suggest that gene introgression from MON 88702 to native populations of *G. tomentosum* is highly unlikely.

Finally, it is important to note that the EPA imposes geographical restrictions on the sale and distribution of cotton with insecticidal traits in order to prevent possible gene flow to wild populations of *Gossypium* species (Wozniak and Martinez 2011). As with other Bt cotton registrations, cultivation of MON 88702 is prohibited or severely restricted in Puerto Rico, the US Virgin Islands, Hawaii, and the southern half of Florida (US-EPA 2018a, 2019). At the time of writing, registration for commercial sale is under review by US-EPA.

In summary, the likelihood of MON 88702 hybridizing with cultivated, wild or feral cotton is low due to the predominance of self-pollination, geographic isolation, and other reproductive barriers. Furthermore, the EPA prohibits commercial use and large-scale cultivation of Bt cotton in areas where wild populations are present. The introduced genetic material in MON 88702 does not cause any major changes in the phenotype of cotton plants other than the intended expression of the mCry51Aa2 protein. Thus, the engineered traits in MON 88702 cotton is unlikely to cause increased levels of gene flow and introgression from MON 88702 into its sexually compatible relatives. Should outcrossing from MON 88702 to *G. barbadense* or *G. tomentosum* occur, transgene introgression would still require the establishment of hybrid progeny in subsequent

generations. In the absence of human aid, the transgenic material in MON 88702 cotton is unlikely to confer a selective advantage on any resulting hybrid progeny.

Potential for enhanced weediness of recipients after hybridization and/or introgression

As discussed in the previous section, the introduced genetic material in MON 88702 does not confer or enhance weedy characteristics of cultivated cotton. Should gene flow and/or introgression from MON 88702 to its sexually compatible species occur, the introduced genetic materials are unlikely to cause enhanced weediness of the recipient plants. Thus, APHIS has determined that any adverse consequences of gene flow and/or introgression from MON 88702 to wild relatives or weedy species in the U.S. and its territories are highly unlikely compared to cultivated non-transgenic cotton.

Based on the information presented in the petition and in relevant literature, APHIS has reached the following conclusions. The genetic modification in MON 88702 cotton is not expected to increase the potential for gene flow, hybridization and/or introgression to occur to sexually compatible taxa compared to the nontransgenic recipient or other varieties of the crop commonly grown. Gene flow, hybridization and/or introgression of genes from MON 88702 to other sexually compatible relatives, including wild, weedy, feral or cultivated species in the U.S. and its territories is not likely to occur. Therefore, MON 88702 cotton is not expected to increase the weed risk potential of other species with which it can interbreed in the U.S. and its territories.

H. Potential Changes to Agriculture or Cultivation Practices

APHIS assessed whether significant changes to agricultural or cultivation practices from adoption of MON 88702 are likely to impact plant diseases or pests or their management, including any APHIS control programs. This includes consideration of any changes in pesticide applications, tillage, irrigation, harvesting, etc. as they relate to plant pests and diseases.

Due to the function of the introduced protein in MON 88702, the only anticipated change to agricultural and cultivation practices due to its adoption would be regarding insecticide applications. There is already widespread adoption of herbicide tolerant and Lepidoptera resistant cotton varieties across the US cotton belt (USDA-NASS 2019b). For control of Lygus and thrips, Graham and Stewart (2018) observed an average reduction of 1.25 insecticide sprays per growing season. Overall, a general decrease in insecticide sprays could be expected, but it should be noted that MON 88702 does not eliminate the need for insecticides to control Lygus (or thrips) (Graham and Stewart 2018; Akbar et al. 2019). On the other hand, if adoption of MON 88702 causes resurgence of any secondary pests, due to reduced competition by target pests, by a reduced efficacy of the natural enemy community, or even reduced opportunistic predation by thrips (Trichilo and Leigh 1986; D'Ambrosio et al. 2020) or Lygus (Cleveland 1997; Rosenheim et al. 2004), then insecticide sprays may not decrease as expected. However, there is no reason to anticipate that insecticide sprays will increase above what is applied currently.

In conclusion, the introduction of MON 88702 will likely result in at least some reduction in usage of insecticides. However, due to incomplete control of *Lygus* and thrips, in addition to the presence of other cotton pests not affected by mCry51Aa2, insecticide usage will not be eliminated from cotton production practices. No other changes to agricultural or cultivation practices of cotton from adoption of this crop are anticipated. Therefore, no impact on plant diseases or pests or their management is likely to occur.

I. Potential Impacts from Transfer of Genetic Information to Organisms with which MON 88702 Cotton Cannot Interbreed

APHIS examined the potential for the new genetic material inserted into MON 88702 to be horizontally transferred without sexual reproduction to other organisms and whether such an event could lead directly or indirectly to disease, damage, injury or harm to plants, including the creation of new or more virulent pests, pathogens, or parasitic plants. The horizontal gene transfer between unrelated organisms is one of the most intensively studied fields in the biosciences since 1940, and the issue gained extra attention with the release of transgenic plants into the environment (Dröge et al. 1998). Potential risks from stable horizontal gene transfer (HGT) from genetically engineered organisms to another organism without reproduction or human intervention were recently reviewed (Keese 2008). Mechanisms of HGT include conjugation, transformation and transduction, and other diverse mechanisms of DNA and RNA uptake and recombination and rearrangement, most notably through viruses and mobile genetic elements. HGT has been a major contributor to the spread of antibiotic resistance amongst pathogenic bacteria; emergence of increased virulence in bacteria, eukaryotes and viruses; and, in the long run, to major transitions in evolution.

Potential for horizontal gene transfer to bacteria, fungi, or invertebrates

MON 88702 cotton has one gene derived from *Bacillus thuringiensis* and nonfunctional border sequence from the disarmed vector *Agrobacterium tumefaciens*. Horizontal gene transfer and expression of DNA from a plant species to bacterial, fungal or invertebrate 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) and with invertebrates as plant pests, there are almost no evolutionary examples of HGT from eukaryotes to bacteria or from plants to fungi or invertebrates (Keese 2008). Examples of HGT between eukaryotes and fungi primarily involve gene acquisition or transfer by fungi to or from other distantly related fungi or bacteria (Keeling and Palmer 2008; Keese 2008) and HGT between plants and fungi is extremely rare (Richards et al. 2009). Examples of HGT between plants and invertebrates are also extremely rare, and most examples of HGT in insects involve acquisition of genes from their pathogens or endosymbionts (Keese 2008; Zhu et al. 2011; Acuna et al. 2012).

Horizontal transfer from and expression in bacteria of the foreign DNA inserted into the nuclear genome of the GE plant 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* (Wood et al. 2001; Kaneko et al. 2002). There is no evidence that these organisms contain genes derived from plants. HGT from plants to bacteria is a very low frequency event, primarily because functional and selective barriers to HGT increase with genetic distance (Keese 2008). 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; EFSA 2009; Koonin et al. 2011). Third, transgene DNA promoters and coding sequences are optimized for plant expression, not prokaryotic bacterial expression. Thus, even if horizontal gene transfer occurred, proteins corresponding to the transgenes are not likely to be produced. Fourth, both the FDA (1998) and the European Food Safety Authority (2009) have 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 very rare or remote.

Potential for horizontal gene transfer to viruses

The GE crop contains an enhancer sequence from figwort mosaic virus and a terminating sequence from cauliflower mosaic virus; both are noncoding sequences. APHIS also considered whether horizontal transfer of DNA from the GE plant to plant viruses was likely to occur and would lead to the creation or selection of plant viruses that are more virulent or have a broader host range. This issue has been considered before by other science review panels and government regulatory bodies (EPA-FIFRA-SAP 2006; Keese 2008). HGT is not unusual among plant viruses; however this is generally limited to exchange between viruses present in the same host organism in mixed infections, and most commonly involves homologous recombination, relying on sequence similarity at the point of crossover (Keese 2008). HGT from virus sequences engineered into plants has been demonstrated with infecting or challenge viruses, including both DNA viruses (e.g. geminiviruses which replicate in the nucleus)(Frischmuth and Stanley 1998) and RNA viruses (which typically replicate in the cytoplasm); however most have been under conditions that favor recombination to restore a defective virus (Fuchs and Gonsalves 2007; Keese 2008; Thompson and Tepfer 2010). Populations of recombinants between virus transgenes expressed in transgenic plants infected with related viruses are similar to recombinants found in mixed infections of the same viruses in nontransgenic plants, indicating that there was no novel recombination mechanism in the transgenic plants and no increased risk is expected over what is expected from mixed infections (Keese 2008; Turturo et al. 2008). Nonhomologous recombination in HGT among viruses or between virus transgenes and infecting viruses can occur, but frequently results in gene deletions which can result in nonviable viruses (Morrone et al. 2013). Depending on the particular virus and sequences involved, various hot-spots for recombination have been found in both coding and noncoding regions, and strategies implemented in design of transgenes to avoid recombination have been suggested. No recombinant or undesirable viruses with new properties have been detected for over at least 8-10 years in field tests or during commercial growth of deregulated virus resistant plum, squash, or papaya engineered with genes from viruses that have been deregulated in the U.S. (Fuchs and Gonsalves 2007).

Potential for horizontal gene transfer to parasitic plants

Evidence for HGT from plants to other plants is limited to two specific scenarios: (1) exchange of genes between a parasitic plant and its host; and (2) exchange of genes between cells of two plants living in close proximity, such as in a graft junction. In both cases, this type of HGT requires physical contacts between the two plants. Most cases of HGT in plants involve transfer of mitochondrial genomes, which are primarily maternally inherited in plants (Barr et al. 2005), to other mitochondria genomes, and mostly involve parasitic plants and their hosts (Richardson and Palmer 2007). Recently, (2010) through a comparative genomics analysis implicated HGT for the incorporation of a specific genetic sequence in the parasitic plant purple witchweed (*Striga hermonthica*) from its monocot host plant. According to this study, the incorporation of the specific genetic sequence (with an unknown function) occurred between sorghum and purple witchweed. However, this HGT occurred before speciation of purple witchweed and related cowpea witchweed (*S. gesnerioides*) from their common ancestor. Furthermore, *S. hermonthica* is not found in the U.S. and *S. asiatica*, another related parasite of cereal crops, is only present in North Carolina and South Carolina (USDA-NRCS). More recent studies demonstrated that in a few parasitic species of the Rafflesiaceae family, out of several genetic sequences examined, about 2.1% of nuclear (Xi et al. 2012) and 24–41% of mitochondrial (Xi et al. 2013) gene transcripts appeared to be acquired from their obligate host species. However, all the above-mentioned instances of HGT between parasitic plants and their hosts were reported to be of ancient origins, on an evolutionary time scale spanning thousands to millions of years ago. Furthermore, in the GE crop, the DNA sequences were inserted into the nuclear genome, not the mitochondrial genome.

If the GE plant becomes infected by a parasitic plant or is naturally grafted to another plant, there is a very low probability that HGT could result in the other plant acquiring DNA from the GE plant. However, in both scenarios this newly introduced DNA would likely reside in somatic cells, and with little chance of reaching the germ cells, this introduced DNA could not persist in subsequent generations unless the recipient plant reproduced asexually from the affected cells.

Based on the above analysis APHIS therefore concludes that HGT of the new genetic material inserted into the GE plant to other organisms is highly unlikely, and is not expected to lead directly or indirectly to disease, damage, injury or harm to plants, including the creation of new or more virulent pests, pathogens, or parasitic plants.

J. Conclusion

APHIS has reviewed the information submitted in the petition, supporting documents, public comments in response to Federal Register notices concerning this petition, and other relevant information to assess the plant pest risk of MON 88702 compared to the unmodified variety from which it was derived. APHIS concludes that MON 88702 is unlikely to pose a greater plant pest risk based on the following findings.

- No plant pest risk was identified from the transformation process or the insertion of new genetic material in MON 88702: the *Agrobacterium* transformation vector was eliminated from the transformed material using antibiotics, and the plant pest sequences inserted do not cause disease or create an infectious agent.
- No increase in plant pest risk was identified in MON 88702 due to unexpected and unrelated effects from expression of the inserted genetic material for control of the target pests.
- Disease and pest incidence and/or damage were not observed to be significantly increased or atypical in MON 88702 compared to the nontransgenic counterpart or other comparators in field trials conducted in growing regions representative of where MON 88702 is expected to be grown. Observed agronomic traits also did not reveal any significant differences that would indirectly indicate that the GE crop event is more susceptible to pests or diseases. Therefore, no plant pest effects are expected on these or other agricultural products, and no impacts are expected to APHIS pest control programs.
- Exposure to and/or consumption of MON 88702 are unlikely to have a significant adverse impact on organisms beneficial to agriculture based on the evaluation of bioassay data on nontarget organisms, supplemented by field data on abundance of nontarget arthropods.
- MON 88702 is no more likely to become a weed than conventional varieties of the crop based on its observed agronomic characteristics, weediness potential of the crop and current management practices available to control MON 88702 as a weed.
- MON 88702 is not expected to increase the weed risk potential of other species with which it can interbreed in the U.S. or its territories. Gene flow, hybridization and/or introgression of inserted genes from the GE crop event to other sexually compatible relatives with which it can interbreed is not likely to occur. Gene flow from MON 88702 would be mitigated primarily because EPA Section 3 pesticide registrations preclude cultivation of PIPs in areas where sexually compatible relatives may be present.
- Significant changes to agricultural or cultivation practices (e.g. pesticide applications, tillage, irrigation, harvesting, etc.) from adoption of the MON 88702 were not identified.
- Horizontal gene transfer of the new genetic material inserted into the GE plant to other organisms is highly unlikely, and is not expected to lead directly or indirectly to disease, damage, injury or harm to plants, including the creation of new or more virulent pests, pathogens, or parasitic plants.

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