BASF Petition (19-317-01p) for Determination of Non-regulated Status for Plant-Parasitic Nematode-Protected and Herbicide Tolerant GMB151 Soybean

OECD Unique Identifier: BCS-GM151-6

Final Plant Pest Risk Assessment

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A. Introduction

BASF Corporation (hereafter referred to as BASF) has submitted a petition to the Animal and Plant Health Inspection Service (APHIS) of the United States Department Agriculture (USDA) seeking a determination of non-regulated status for soybean (*Glycine max* (L.) Merr.), event GMB151 (OECD Unique Identifier BCS-GM151-6) developed using genetic engineering to be protected against a plant parasitic nematode and for resistance[1] to HPPD-inhibitor herbicides (WSSA 1998) that is unlikely to pose a greater plant pest risk than the unmodified organism from which it was derived and, therefore, should no longer be regulated under the APHIS' 7 Code of Federal Regulations (CFR) part 340. This petition was assigned the number 19-317-01p and is hereafter referenced as BASF 2019. Under the authority of the plant pest provisions of the Plant Protection Act (PPA), as amended (7 U.S.C. 7701et seq.) the regulations in 7 CFR part 340, "Movement of Organisms Modified or Produced Through Genetic Engineering," regulate, among other things, the importation, interstate movement, or release into the environment of organisms modified or produced through genetic engineering that are plant pests or that there is a reason to believe are plant pests. This plant pest risk assessment (PPRA) was conducted to determine if GMB151 soybean is unlikely to pose a plant pest risk.

The petition for non-regulated status described in this PPRA is being evaluated under the version of the regulations effective at the time that it was received. The Animal and Plant Health Inspection Service (APHIS) issued a final rule, published in the Federal Register on May 18, 2020 (85 FR 29790-29838, Docket No. APHIS-2018-0034) [2], revising 7 CFR part 340; however, the final rule is being implemented in phases. The new Regulatory Status Review (RSR) process, which replaces the petition for determination of non-regulated status process, became effective on April 5, 2021 for corn, soybean, cotton, potato, tomato, and alfalfa. The RSR process is effective for all crops as of October 1, 2021. However, "[u]ntil RSR is available for a particular crop APHIS will continue to receive petitions for determination of non-regulated status for the crop in accordance with the [legacy] regulations at 7 CFR § 340.6." (85 FR 29815). This petition for a determination of non-regulated status is being evaluated in accordance with the regulations at 7 CFR § 340.6 (2020) as it was received by APHIS on 11/13/2019.

GMB151 soybean was developed by transformation of explants of the soybean variety Thorne, using *Agrobacterium tumefaciens* mediated transformation with the transformation vector pSZ8832 (BASF 2019 pp.19, 32-39). The GMB151 soybean

[1] BASF has also described the phenotype of GMB151 soybean as "herbicide tolerant" and historically APHIS has also referred to modified plants with reduced herbicide sensitivity as herbicide tolerant. However, the phenotype would fall under the Weed Science Society of America (WSSA) definition of "herbicide resistance" since GMB151 soybean has an "inherited ability to survive and reproduce following exposure to a dose of herbicide normally lethal to the wild type." By the WSSA definition, "resistance (to an herbicide) may be naturally occurring or induced by such techniques as genetic engineering or selection of variants produced by tissue culture or mutagenesis." Herbicide tolerance, by the WSSA definition, only applies to plant species with an "inherent ability to survive and reproduce after herbicide treatment. This implies that there was no selection or genetic manipulation to make the plant tolerant; it is naturally tolerant."

[2] To view the final rule, go to www.regulations.gov and enter APHIS-2018-0034 in the Search field.

contains non-coding regulatory sequences from plant pest organisms listed in 7 CFR 340.2, including the *Cauliflower mosaic virus* (CaMV) 35S promoter and terminator regions (Kay et al. 1987; Sanfacon et al. 1990), and a leader sequence derived from *Tobacco etch virus* (TEV) (Allison et al. 1985; BASF 2019, p. 32) and right and left border T-DNA repeats from *A. tumefaciens,* a plant pest. Portions of the introduced genetic material were derived from plant pest organisms listed in 7 CFR 340.2. Therefore, GMB151 soybean is considered a regulated organism under APHIS regulations at 7 CFR part 340. BASF has conducted field trials in the U.S. of GMB151 soybean as a regulated organism under APHIS authorizations since 2013 (BASF 2019 Appendix 1, Table A1, p. 131). Field trials were conducted for research, development, breeding, and seed multiplication.

Potential impacts discussed in this plant pest risk assessment are those that pertain to plant pest risk associated with GMB151 soybean 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 GMB151 soybean is unlikely to pose a greater plant pest risk than the unmodified organism from which it was derived. APHIS regulations in 7 CFR 340.6(c) specify the information needed for consideration in a petition for non-regulated status. APHIS will assess information submitted by the applicant about GMB151 soybean 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 modified organism on non-target organisms; weediness of the modified organism; 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; 80 FR 60414 2015; USDA FDA EPA 2017). 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.

Under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) (7 U.S.C. §136 *et seq*), EPA regulates the distribution, sale, use and testing of pesticidal substances produced in plants and microbes, including those pesticides 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 §301 *et seq.*). 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 label requirements for pesticides and devices (40 CFR part 156). Other applicable EPA regulations include Pesticide Registration and Classification Procedures (40 CFR part 152), Experimental Use Permits (40 CFR part 172) and Procedures and Requirements for Plant Incorporated Protectants (PIPs) (40 CFR part 174).

As cited in the petition subject of this PPRA, the Cry14Ab-1 protein produced by GMB151 is a plant incorporated protectant (PIP) and is regulated as a pesticide by the US EPA. An experimental Use Permit (EUP), as described under Section 5 of Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), is in effect for Cry14Ab-1 and the genetic material responsible for its production in GMB151 soybean (BASF 2019, p. 17). The petition also mentions that BASF submitted an application for a FIFRA Section 3 seed increase registration to the U.S. EPA on November, 2018 for Cry14Ab-1 and the genetic material responsible for its production in GMB151 soybean. A temporary exemption from the requirement for a tolerance was granted to Cry14Ab-1 in conjunction with the previously mentioned EUP. A petition for a permanent exemption from the requirement for a tolerance for the Cry14Ab-1 protein when expressed in soybean was submitted to the U.S. EPA on November, 2018 in conjunction with the Section 3 registration submission (BASF 2019, p.17). The US EPA has granted a permanent exemption from the requirement of a tolerance for the residues of the HPPD-4 protein derived from the 4-hydroxyphenylpyruvate dioxygenase in or on all food commodities, when used as a plant incorporated inert ingredient. The EPA concluded based on the available data that this enzyme does not show evidence of toxicity, the source is not allergenic, nor there is any significant similarity to known toxins and allergens. The HPPD-4 proteins are readily digested in gastric fluids and therefore cumulative, chronic and acute effects are not likely (40 FR 260086 2017).

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 and a more comprehensive voluntary consultation process prior to commercial distribution of food or feed (57 FR 22984 1992). BASF Corporation has initiated a consultation with the FDA that included molecular, composition, and nutrition data, as well as other food and feed safety assessment data related to plant-parasitic nematode-protected GMB151soybean.

B. Development of the GMB151 Soybean

Glycine max (L) Merr., the cultivated soybean, is an economically important diploidized tetraploid plant in the family Leguminosae. The genus *Glycine* has two subgenera: *Soja* and *Glycine*. The subgenus *Soja* includes two species *Glycine max* and *Glycine soja*; whereas the subgenus *Glycine* includes 26 wild perennial species indigenous to Australia, Asia and other countries in Oceania (Chung and Singh 2008; Sherman-Broyles et al. 2014). *Glycine max* is believed to have been domesticated in East Asia from its wild

progenitor *G. soja* between the 17th and 11th century B.C. (Hymowitz 1970; Hymowitz and Newell 1981; Sedivy et al. 2017). No wild relatives of *G. max* are found growing naturally in North America, but they grow in many parts of Asia.

The soybean plant is an erect, bushy herbaceous annual that can grow to 1.5 meters in height. It is propagated commercially by seed, and three types of growth habit are found among the different cultivars: determinate, semi-determinate and indeterminate. Determinate and semi-determinate cultivars belong to different maturity groups, which are grown in different parts of the United States. Soybean varieties are not frost tolerant and do not survive freezing winter conditions (OECD 2000). The root system consists of a taproot and large number of lateral roots that establish a symbiotic relationship with *Bradyrhizobium japonicum*, a nitrogen fixing bacterium in the soil, through formation of root nodules (Chung and Singh 2008).

Soybean is grown as a crop worldwide, and the United States and Brazil are the world's leading soybean producers, followed by Argentina, China, India, and other countries (USDA-FAS 2017). Soybeans are grown commercially for the seeds which are used for food and feed and have approximately 38% protein content and 18% oil content. Most of the soybeans produced in the world are processed or crushed into soybean meal and oil. Nearly all soybean meal (98%) is used in livestock and aquaculture feeds. On the other hand, 95% of the oil fraction is consumed by humans as edible oil, and the rest is used for industrial purposes (Chung and Singh 2008; Hartman et al. 2011).

Soybean was first introduced in the United States in 1765 (Hymowitz and Harlan 1983), and soybean seed production was initially low amounting to 1,600 acres in 1909. Soybeans are grown mostly in the Midwest as shown in Figure 1 (USDA-NASS 2019), and in 2018 a total of 89,000 million acres were planted nationwide with production of over 4.5 million bushels indicated in Figure 2 (USDA-NASS 2020).

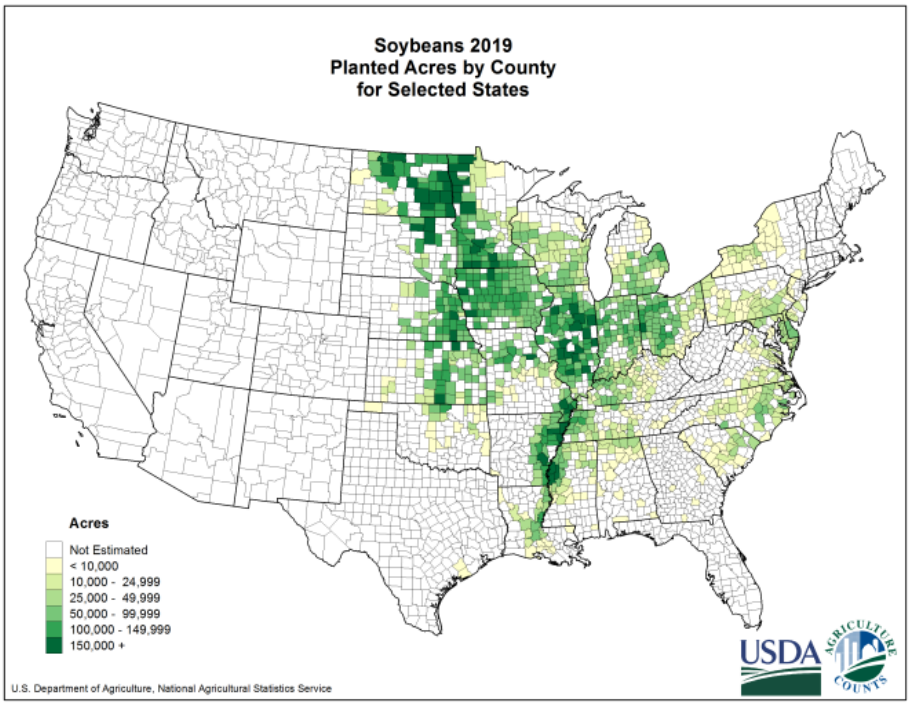

Figure 1. Soybean production areas in the U.S. (USDA-NASS 2019)

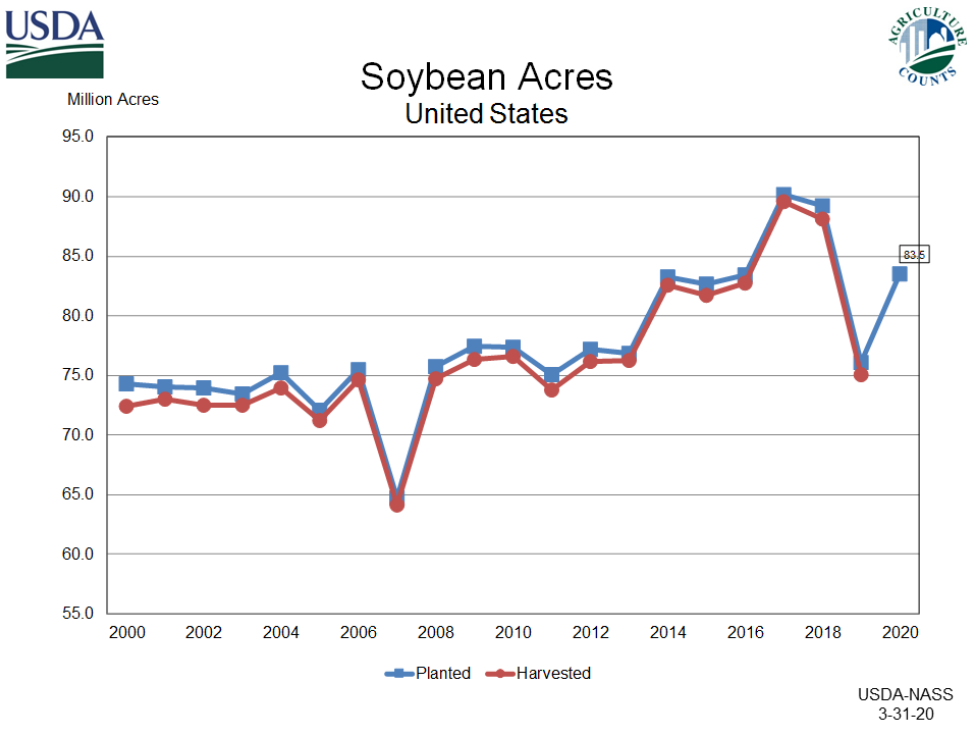

Figure 2. Soybean acreage by year in the U.S. (USDA-NASS 2020)

Despite the continuous soybean yield increase over the years, soybean production faces multiple challenges by a variety of both biotic and abiotic stress factors. Typical abiotic stress factors include salinity, non-optimal temperatures, drought, flooding, and poor soil nutrition, etc. (Chung and Singh 2008). Among the disease-causing agents of soybean, *Heterodera glycines*, the soybean cyst nematode (SCN), is of major economic importance on soybean production worldwide. It is considered the most damaging pathogen of soybeans in United States and Canada, and has been found in every soybean producing state in the United States (Tylka and Marett 2014).

Crop rotation with non-host plants and use of resistant cultivars are management practices that can be used in *H. glycines* infested fields to reduce the population in the soil, or to increase soybean yields. Growers are advised to rotate sources of resistance. There are hundreds of SCN resistant soybean cultivars, and most of the resistance is derived from Plant Introduction (PI) 88788, a few cultivars have resistance derived from PI 548402 (Peking) and PI 437654. The level of resistance in the different cultivars varies, and in order to reduce selection pressure for different *H. glycines* populations, growers are advised to rotate sources of resistance.

GMB151 soybean was developed through *Agrobacterium* mediated transformation of the soybean cultivar Thorne using the vector pSZ8832, containing *cry14Ab-1* and *hppdPf-4Pa* gene cassettes. The *cry14Ab-1* gene was derived from *Bacillus thuringiensis* and was optimized for plant expression. The Cry14Ab-1 protein, is a member of crystal type (Cry) protein family that demonstrates specific toxicity towards nematodes. Expression of Cry14Ab-1 in GMB151 is intended to confer resistance to SCN. The *hppdPf-4Pa* gene was derived from *Pseudomonas fluorescens* and its sequence was modified to generate a protein with reduced HPPD-inhibitor herbicide binding efficacy. Expression of the modified 4-hydroxyphenylpyruvate dioxygenase (HPPD-4), is intended to confer tolerance to HPPD inhibitor herbicides such as isoxaflutole.

According to the petition, GMB151 soybean expressing Cry14Ab-1 will be combined with commercially available varieties that have resistance to SCN, to extend the durability of resistance derived from GMB151 and from native resistance to SCN found in resistant cultivars. GMB151 was also engineered to express a modified gene that confers resistance to HPPD inhibitor herbicide intended to provide growers with an additional HPPD-inhibitor herbicide option (BASF 2019 p. 16).

BASF conducted field activities with GMB151 soybean under USDA authorizations for research, development, regulatory, breeding and seed multiplication from 2013 to 2019. There were no reports of unusual or unexpected phenotypes, effects on NTOs, susceptibility to plant pests, or other unexpected interactions with the biotic or abiotic environment (BASF 2019, p. 131, Table A1.1). GMB151 was also field tested in Brazil for efficacy against nematodes causing disease of soybean (BASF 2019).

Based on soybean biology (Hymowitz and Newell 1981; OECD 2000; Chung and Singh 2008; Sedivy et al. 2017) and the data presented (BASF 2019), APHIS concludes that the GMB151soybean was developed in a manner common to other soybean and crops

modified using *Agrobacterium*-mediated transformation (USDA-APHIS 2017). APHIS believes the use of the non-modified parental line Thorne and other reference varieties as comparators is sufficient to determine that GMB151 soybean is not substantially different from its non-modified parental line and non-modified conventional soybean varieties (USDA-APHIS 2017).

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 DNAs and their 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 modified 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 GMB151 soybean relative to the unmodified soybean variety Thorne and other soybean commercial varieties as described in the petition. The assessment encompasses a consideration of the expressed Cry14Ab-1 and HPPD-4 proteins and any observed or anticipated effects on plant metabolism, including, e.g. any relevant changes in levels of metabolites, anti-nutrients, or nutrients in forage and grains derived from the GMB151 compared to those in the non-modified counterpart Thorne and nine non-modified reference varieties.

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 GMB151 soybean; or for expression of inserted DNAs, new proteins or enzymes, or changes in metabolism to affect plant pest or diseases, non-target 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

As described in the petition, GMB151 soybean was developed using disarmed *Agrobacterium tumefaciens* mediated transformation of explants from the soybean variety Thorne with the transformation vector pSZ8832. Following transformation, the explants were placed on selection medium supplemented with tembotrione and ticarcillin to select for transformed cells and eliminate *A. tumefaciens* (BASF 2019 p. 19).

The vector pSZ8832 is approximately 14.4 Kbps and contains two gene expression cassettes *cry14Ab-1* and *hppdPf-4Pa* delineated by right (RB) and left border (LB) sequences of T-DNA of *Agrobacterium tumefaciens* as well as backbone sequences outside the T-DNA borders (BASF 2019 pp 19-22, Figure 1 p. 20, Table 1, p. 21-22)

The *cry14Ab-1* gene cassette contains the genetic elements:

- Pubi10At: sequence including the promoter region of ubiquitin-10 gene of *Arabidopsis, thaliana* (Grefen et al. 2010);
- *cry14Ab-1.b*: coding sequence of the delta-endotoxin gene of *Bacillus thuringiensis*;
- T35S: sequence including the 3' untranslated region of the 35S transcript of the Cauliflower mosaic virus (Sanfacon et al. 1990).

The *hppdPf-4Pa* gene cassette contains the genetic elements:

- P2x35S: sequence including the double enhanced promoter regions of the Cauliflower mosaic virus 35S genome transcript (Kay et al. 1987);
- Ltev: sequence including the leader sequence of the Tobacco etch virus genomic RNA (Allison et al. 1985);
- *TPotpY-1Pf*: coding sequence of an optimized transit peptide derivative containing sequences of the RuBisCO small subunit genes of *Zea mays* and *Helianthus annuus* (Lebrun et al. 1996);
- *hppdPf-4Pa*: coding sequence of a variant 4-hydroxyphenyl pyruvate dioxygenase gene of *Pseudomonas fluorescens* (Poree et al. 2014);
- T35S: sequence including the 3' untranslated regions of the 35S transcript of the Cauliflower mosaic virus (Sanfacon et al. 1990).

Although some of the genetic elements used in the constructs were derived from plant pests, they do not encode a plant pest or an infectious agent. The T-DNA left and right border sequences, the leader sequence from Tobacco etch virus and the Cauliflower mosaic virus 35S promoter and terminator are derived from plant pathogens. However, none of the regulatory elements, or the left and right border elements are known to cause plant diseases. Detailed descriptions of the genetic elements in the inserted DNA and references for each element are found in the petition (BASF 2019, pp. 21-22, Table 1, p.20 Figure 1).

BASF performed molecular characterization of the genetic modifications in GMB151 soybean using next generation sequencing (NGS), junction sequence analysis (JSA), Sanger sequencing and bioinformatics analysis. Genomic DNA prepared from GMB151 seeds was used to characterize inserted sequences using NGS/JSA. The non-modified variety Thorne was used as the negative control sample. The positive control and sensitivity control samples were the non-modified counterpart Thorne supplemented with plasmid pSZ8832 DNA. Whole genome sequencing was performed using the Illumina HiSeq platform 2500 technology. Median genome sequencing coverage was examined by the alignment of the read to the lectin gene, a known single copy locus, and was higher than 75-fold (BASF 2019 p.27, p.196).

Bioinformatics analysis of NGS/JSA and Sanger sequencing results demonstrated that GMB151 soybean contains one copy of the T-DNA insert, without rearrangements, at a single insertion site. The *cry14Ab-1* gene cassette is complete, and the *hppdPf-4Pa* gene cassette lacks 482 bp at the 5'end of the P2x35S promoter. Upon transformation, 63 bp from the non-modified counterpart parental variety Thorne were replaced with 7,498 bp

of inserted sequences between the GMB151 T-DNA sequence and the 3' flanking genomic region, 7,459 bp of T-DNA sequence and 39 bp of filler DNA. Of the filler DNA, 21 bp show sequence identity to ORIpVS1 in the pSZ8832 vector backbone, and 17 bp show sequence identity to soybean genomic DNA in the 3' flanking genomic region (BASF 2019, pp. 29-30).

Additionally, bioinformatics analysis further demonstrated that the GMB151 insertion is located on chromosome 7, in the 3' untranslated region of a putative endogenous gene, annotated as BON1-associated protein 1 BAP1-like protein. In *Arabidopsis thaliana*, the BAP1 protein has a function in a signal transduction cascade (BASF 2019, p. 35). Based on an assessment of agronomic and compositional analysis of GMB151 with the non-GE counterpart, there were no unexpected or unintended effects, and no impact on GMB151 agronomic performance or on the nutritional value of forage and grain. BASF concluded that there are no reasons to assume an effect on plant pest risk due to interruption of the putative BAP1-like locus (BASF 2019 , p.35).

BASF demonstrated using NGS/JSA that the GMB151 insertion locus is stably maintained across five breeding generations The segregation ratios of the insert over five segregating generation confirmed stable and predictable inheritance according to Mendelian inheritance principles (BASF 2019, pp. 33-34).

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

GMB151 soybean expresses two recombinant proteins, the Cry14Ab-1 protein, and a modified 4-hydroxyphenylpyruvate dioxygenase (HPPD-4).

<u>Cry14Ab-1 protein</u>

The Cry14Ab-1 protein expressed in GMB151 soybeans consists of 1,185 amino acids with an expected molecular mass of 131.1 kDa. The *Cry14Ab-1* gene was derived from *Bacillus thuringiensis* (Bt) and was optimized for plant expression. The protein was found in bioassays to be active against *Caenorhabditis elegans*, and GMB151 plants expressing the Cry14AB-1 protein were found to be more resistant to *Heterodera glycines*, the soybean cyst nematode (SCN) than the non-modified parental counterpart (BASF 2019, p. 156).

Bacillus thuringiensis is a Gram positive, soil bacterium that produces spores containing crystal protein inclusions during the sporulation phase (Sanahuja et al. 2011; Bravo et al. 2012). The Cry proteins are encoded by *cry* genes carried on plasmids present in different strains of *B. thuringiensis* that produce different types of Bt toxins (Reyes-Ramirez and Ibarra 2008). These toxins have been found to be selectively active against insects, nematodes, mites and protozoa (Bravo et al. 2012). The insecticidal Cry proteins have specificity against a limited number of species in certain taxonomic orders such as Lepidoptera and Coleoptera, and have not been shown to have toxicity against other organisms including humans (Sanahuja et al. 2011; Bravo et al. 2012). The Cry14Ab-1 protein, demonstrates specific toxicity towards nematodes and is homologous to Cry14Aa1 with 87% identity (BASF 2019).

HHPD-4 protein

The modified 4-hydroxyphenylpyruvate dioxygenase, HHPD-4, expressed in GMB 151 soybean consists of 358 amino acids with an expected molecular mass of 40.3 kDa. The *hppdPf-4Pa* gene was mutated at four locations to introduce four amino acid changes, to generate HHPD-4 with reduced HPPD-inhibitor herbicide binding efficacy. HPPD-4 confers tolerance to HPPD inhibitor herbicides such as isoxaflutole. The gene for HPPD-4 was derived from *P. fluorescens*, a Gram-negative, aerobic bacterium commonly found in soil in the plant rhizosphere and phyllosphere, in water, and animals (OECD 1997).

HPPD catalyzes the conversion of 4-hydroxyphenylpyruvate to homogentisate, an important step in the degradation of aromatic amino acids, and is important in the catabolism of tyrosine and phenylanine. Homogentisate serves as a precursor in the biosynthesis of plant plastoquinones and tocopherols, lipid soluble compounds present in higher plant chloroplasts, and essential in photosynthetic transport chain and antioxidative systems. HPPD inhibition results in the disruption of the biosynthesis of carotenoids, leading to death of the plants (Fritze et al. 2004).

The EPA concluded based on the available data that HPPD-4 does not show evidence of toxicity, nor there is any significant similarity to known toxins and allergens, it is rapidly degraded in simulated gastric fluid and that cumulative, chronic and acute effects are not likely (40 FR 260086 2017).

Expression of new proteins

Protein expression levels of Cry14Ab-1 and HHPD-4 were determined using protein specific enzyme-linked immunosorbent (ELISA) assays validated for each protein. Tissue samples were collected from field-grown GMB151 soybean plants from field sites representative of the commercial production of soybeans in respect to cultural practices, soil type and climate. Samples were taken from leaves, roots, flowers, forage, whole plants, and grain for protein quantitation at different soybean growth phases during 2016 using the BBCH scale (Munger et al. 1997; BASF 2019). Field plots were either not treated with herbicide, or treated with the trait specific herbicide, isoxaflutole, before emergence at growth stage BBCH 00 (BASF 2019).

Mean expression level of Cry14Ab-1 in GMB151 soybean tissues was lower in roots than in leaves at all tested growth stages, and lower than in forage and grain. Highest mean levels in leaves and roots were seen at vegetative stage BBCH 16-17, and the lowest mean levels in roots was seen at the flowering stage BBCH 60-66. For HPPD-4 in treated and non-treated GMB151 soybean tissues the highest mean protein levels were demonstrated in leaves at the early vegetative stage BBCH 13-14 (BASF 2019, pp. 39-45).

Recombinant Cry14Ab-1 and HPPD-4 proteins were expressed in *B. thuringiensis* and in *Escherichia coli* to generate sufficient quantities for use in the safety assessment studies. The bacterially expressed proteins were demonstrated to be equivalent to the proteins expressed in GMB151 in a panel of analytical tests, including Coomassie stained sodium

dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), western blot analysis, glycostaining analysis, ultra-performance liquid chromatography-ultra violet-mass spectrometry (UPLC-UV-MS), N-terminal sequence analysis by Edman degradation and peptide mapping. Additionally, functional equivalence of plant and bacterially expressed Cry14Ab-1 was also demonstrated in a bioassays using *C. elegans* (BASF 2019, pp. 46-62).

Potential new open reading frames (ORF)

BASF performed bioinformatics analysis of the sequence of the insertion in GMB151, and concluded that there were no *in silico* toxicological or allergenic findings associated with potential open reading frames (BASF 2019, p. 36).

Compositional analysis

BASF conducted composition analyses of forage and grain samples as part of the comparative assessment between GMB151 and the non-modified counterpart Thorne. Nine non-modified reference varieties representing the existing natural variability in soybeans were also used as comparators (BASF 2019, p. 79). Samples for analyses were collected from eight field trials conducted in 2017. The selected sites are representative of environments where GMB 151 soybean is likely to be grown commercially. Forage samples were harvested at pod formation stage BBCH 71-78 and analyzed for proximates, fiber, calcium, and phosphorous. Grain samples were harvested at grain maturity stage BBCH 89-99 and were analyzed for proximates, fiber, amino acids, fatty acids, minerals, vitamins and anti-nutrients

The results of the compositional analysis and of the comparative assessment, demonstrated that GMB151 soybean forage and grain are comparable to the non-modified counterpart Thorne and to the nine non-modified reference varieties, and no biologically significant differences were found (BASF 2019). Based on these results, it can be concluded that GMB151 soybean is compositionally and nutritionally equivalent to conventional soybean varieties. There are no observed or anticipated unintended metabolic composition changes in the GMB151 soybean that could impart any new plant pest or disease risk than non-modified soybean varieties.

The expression of the inserted DNAs and the resulting phenotype of GMB151soybean are consistent with the stability/inheritance of the introduced genetic material. The ORF analysis showed no evidence of new ORFs or any unintended effects resulting from the insertion of the genetic materials. Based on compositional studies, characteristics of the expressed proteins, and results of field trials, as well as the previous citations and petitions for modified organisms granted non-regulated status with similar genes and gene products that have a history of safe use and have not been implicated in disease or pest issues, the gene products Cry14Ab-1 and HPPD-4 in GMB151 soybean are not expected to incur any additional plant pest or increased disease risks (BASF 2019).

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 GMB151 soybean 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 GMB151 soybean is likely to have significantly increased disease and pest susceptibility based on data and observations from field trials and laboratory experiments on specific pest and disease damage or incidence and any agronomic data that might relate to such damage. Impacts or changes are assessed to determine if they would (1) affect the new modified plant 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 the non-modified counterpart (Thorne) 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 (USDA-APHIS 2020).

Soybean is one of the most economically important commodity crops in the United States, but its profitability has been impacted by soybean diseases and pests (Bandara et al. 2020). Disease causing organisms of soybeans include fungi, nematodes, oomycetes, bacteria and viruses. Arthropod pests of soybeans include insects and acari (Hartman et al. 2011). Diseases caused by a combination of more than one pathogen and abiotic factors such as drought will result in greater stress to the plant, and ultimately greater yield losses.

The most economically damaging diseases in soybean in a 21 year period between 1996 and 2016 were soybean cyst nematode (*Heterodera glycine*), charcoal rot (*Macrophomina phaseolina*), and seedling diseases (caused by various organisms including *Fusarium spp*., *Pythium spp*., *Phomopsis spp.*, and/or *Rhizoctonia solani*). The least economically damaging diseases over this time period were bacterial blight, southern blight and soybean rust. It is of note that soybean losses due to soybean rust increased from 2004 to 2016 after the introduction of *Phakopsora pachyrhizi* in the U.S. (Bandara et al. 2020).

Asian soybean rust caused by *P. pachyrhizi* can be a major disease limiting soybean production in the U.S. It can overwinter on alternate hosts in frost free areas and spread

from states bordering the Gulf Mexico to other soybean producing regions (Hartman et al. 2016). *Macrophomina phaseolina* is a soil-borne pathogen with a wide host range and affects roots and stems causing soybean charcoal rot (Smith et al. 2016). Sudden death syndrome (SDS) of soybean is one of the most important soybean diseases in North America, and is caused by *Fusarium virguliforme* (formerly *F. solani f. sp. glycines*), it can lead to yield losses of up to 100%, and is most severe if SCN is present in the soil (Westphal et al. 2018).

Soybeans are affected by viruses such as *Soybean mosaic virus* (SMV) and *Bean pod mottle virus* (BPMV) that cause damage to soybean plants and seed discoloration. Several bacterial species, such as *Pseudomonas savastanoi* pv *glycinea* cause disease on soybeans throughout the United States. Insect pests such as aphids, beetles, mites and stink bugs can also cause considerable economic damage to soybean crops. *Aphis glycines* (Hemiptera: Pentatomidae) is the major insect pest of soybeans and can directly damage the plants through feeding and indirectly by transmission of soybean mosaic virus and other soybean viruses (Hartman et al. 2011; Hartman et al. 2016).

Among the nematodes listed as causing economic losses to soybeans are *Heterodera glycine*, *Meloidogyne spp*, *Rotylenchus reniformis*, *Belonolaimus longicaudatus*, *Helicotylenchus spp*., *Hoplolaimus spp*, *Paratrichodorus spp*, and *Pratylenchus spp*. *Heterodera glycine*, the soybean cyst nematode (SCN) occurs in most soybean producing regions and can become the greatest limiting factor to soybean production. The soybean cyst nematode along with charcoal rot are among the top yield loss causing diseases globally (Bandara et al. 2020).

The soybean cyst nematode is an obligate endoparasite. The adult form, eggs and four juvenile stages comprise its life cycle. The second-stage juvenile is the infective stage, it emerges from the eggs and enters the plant roots where the remaining life stages develop. Nematode feeding on host plants induces the production of the syncytium, a specialized feeding site near the vascular system. Females become sedentary and lemon shaped, and after fertilization by vermiform males produce large numbers of eggs. The egg filled body becomes a tough walled cyst after the death of the nematode. The cyst protects the eggs and can persist in the soil in a dormant state for many years. Infestation with SCN results in an increase of lateral roots and reduction the number of *Rhizobium* nodules (Niblack et al. 2006; OEPP-EPPO 2018). Each female can produce up to 600 eggs, and can remain viable in a non-hatched condition for up to eleven years (Niblack et al. 2006).

Soybean is not a plant pest in the United States according to 7 CFR 340. The genetic modifications of GMB151 soybean, including genetic elements, expression of the gene products and their functions have been summarized above. The *Agrobacterium* strain used in the generation of GMB151 soybean was disarmed and the bacteria were killed with antibiotics during the transformation process. The inserted DNA elements derived from plant pests do not result in the production of infectious agents or disease symptoms in plants. Thus, it is unlikely that GMB151 soybean could pose a plant pest risk.

In its evaluation of phenotypic and agronomic performance of GMB151 soybean in comparison to the non-modified comparator Thorne and nine non-modified varieties

described in Section C above, BASF also evaluated severity of incidence and plant response to biotic (arthropods and disease) stress (BASF 2019 Section 8, p. 97). Observed pests were: caterpillars (Alfalfa, wooly bear, thistle), aphids, armyworms, bean leaf beetles, cutworms, grape colaspis, grasshoppers, green clover worms, Japanese beetles, leafminer, loopers, Mexican bean beetles, soybean skipper, spider mites, stem borers, stink bugs, thrips, and whitefly. The observed diseases were bacterial blight, bacterial pustule, bean pod mottle virus, brown spot, brown stem rot, Cercospora leaf spot, charcoal rot, downy mildew, frogeye leaf spot, Phytophthora blight, Phytophthora root rot, powdery mildew, Rhizoctonia foliar blight, Rhizoctonia rot, rust, Septoria, soybean mosaic virus, soybean vein necrosis virus, sudden death syndrome, target spot, and white mold. No differences between GMB151and the conventional counterpart were found in 264 observations of biotic stress (BASF 2019 Table 42, p. 113 and Table 43, p. 114).

BASF conducted field studies to assess the phenotypic, agronomic, and environmental interaction characteristics of GMB151 soybean. In a combined-site analysis, there were no biologically relevant statistically significant differences between GMB151 soybean and the conventional counterpart Thorne in susceptibility to the observed arthropod pests and diseases encountered in the field studies. GMB151 soybean plants expressing Cry14Ab-1 showed resistance against *Heterodera glycines* in field test evaluations in the United States. Field tests were also carried out in four locations in Brazil for effects on nematodes causing soybean disease and significant losses, *Pratylenchus brachyurus,* the migratory lesion nematode, and *Helicotylenchus spp.*, the spiral nematodes. The GMB151 transgenic soybean trait significantly reduced populations of *Pratylenchus brachyurus* but did not provide efficacy against spiral nematodes relative to the comparator (BASF 2019 p. 162 and 156).

Other than the intended effect on the target pest the plant parasitic nematode *H. glycines*, the introduced genes did not significantly alter the observed insect pest infestation and disease occurrence or resulting damage on GMB151 soybean over the non-modified counterpart Thorne.

The observed agronomic performance and composition data demonstrated that there were neither significantly altered agronomic traits, nor change in composition that would render GMB151 soybean more susceptible to pests and diseases over the non–modified counterpart Thorne or the reference soybean varieties. At all locations in the United States there were no significant differences between GMB151 soybean and the comparator varieties for the pests and diseases investigated (BASF 2019, p. 113 Table 42, p. 114 Table 43). Thus, GMB151 soybean is unlikely to be more susceptible to plant pathogens and insect pests than conventional soybean and is unlikely to pose a greater plant pest risk than the non-modified counterpart Thorne from which it was derived. For this reason, GMB151 is unlikely to differ from conventional soybean 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 Non-target Organisms Beneficial to Agriculture

The GMB151 soybean has been developed using genetic engineering to confer resistance to the plant parasitic nematode *Heterodera glycines* and for herbicide tolerance. APHIS assessed whether exposure or consumption of GMB151 soybean and the plant incorporated protectant (PIP) Cry14Ab-1 would have a direct or indirect adverse impact on species beneficial to agriculture**.** Organisms considered in the petition were representatives or surrogates of the species associated with production of the modified crop in the agricultural environment, while other studies measured beneficial species directly. The assessment includes an analysis of toxicity and specificity of the Cry14A-1 protein, calculation of the exposure of sensitive nontarget organisms in the agricultural environment to the plant expressed Cry14Ab-1, and a study of the effect of exposure to the GMB151 soybean on the soil community of free-living nematodes. It also includes an analysis of the GMB151 soybean compared to the non-modified counterpart (or other comparators) with respect to the following: any biologically relevant changes in the phenotype or substances (e.g. proteins, nutrients, anti-nutrients, metabolites, etc.) produced which may be novel or expressed at significantly altered amounts that are associated with impacts on organisms beneficial to agriculture.

GMB 151 soybean expresses Cry14Ab-1 in all assayed plant tissues (leaf, root, flower, and grain) (BASF 2019), with the highest expression levels in leaves Thus, aboveground beneficial organisms may be exposed to Cry14Ab-1 through direct or indirect consumption (e.g., consuming leaf, flower, or grain tissue or consuming another organism that has previously fed on GMB 151). Belowground beneficial organisms may be exposed to Cry14Ab-1 through direct or indirect consumption or through substrate exposure (e.g., contact with living or dead soybean plant tissue in the soil matrix or breakdown products or leachates of soybean tissue in soil water).

Among Cry proteins, Cry5, Cry6, Cry13, Cry14, Cry21 and Cry55 are reported to have activity against nematodes (Wei et al. 2003; Ruan et al. 2015) The Cry14Ab-1 protein is homologous to Cry14Aa1 with 87% identity, and demonstrates specific toxicity towards nematodes (BASF 2019).

Cry14Ab-1 is part of a subfamily of *B. thuringiensis* crystal proteins known to be toxic to nematodes (Wei et al. 2003; Ruan et al. 2015), and which have unsubstantiated reports of activity against some Coleoptera (Frankenhuyzen 2009). Cry14A proteins have been shown to be toxic to several nematodes, representing phylogenetically diverse groups within the phylum Nematoda, although not all nematodes are susceptible to the protein (Wei et al. 2003). Cry14Ab-1 was shown by the applicant to be toxic to *Caenorhabditis elegans*, a nematode commonly used as a model organism in laboratory assays (BASF 2019). Although direct toxicity was not measured, the applicant showed that GMB151 soybean expressing Cry14Ab-1 reduces populations and reproductive rates of the plant parasitic nematodes *Heterodera glycines* and *Pratylenchus brachyurus* but not of *Helicotylenchus* spp. (BASF 2019). Taken together, this expression pattern of Cry14Ab-1 in the plant and the known activity of the toxin indicate that Cry14Ab-1 may be likely to

be toxic to some but not all free-living soil nematodes, if the free-living nematodes were exposed to high enough doses of the toxin in the environment.

Risks to free-living soil nematodes

BASF addressed safety concerns regarding the impact of GMB151 on free-living nematodes in two ways. To understand the extent that belowground beneficial nematodes are exposed to the toxin, BASF estimated the aerobic degradation rate of Cry14Ab-1 in the soil. To understand whether the presence of Cry14Ab-1 in the soil impacted the nematode community at realistic field exposure levels, BASF assessed the effects of two years of cultivation of soybean with and without the Cry14Ab-1 expression trait from GMB151 and a conventionally-bred nematode resistance trait on the community of free-living nematodes in the soil.

Additionally, the aerobic degradation rate of Cry14Ab-1 was estimated in soils representative of agricultural soil types for soybean cultivation in the U.S., and representing diverse geographical and climatic conditions, and physiochemical soil characteristics. The soil samples were collected from the top layer of the soil from California, Iowa, Kansas and Nebraska. BASF concluded that over 50% of the Cry14Ab-1 protein added to the soil in a water solution degraded in less than 0.5 days at 20ºC (86ºF) (BASF 2019 Appendix 5, p. 168).

An assessment of the potential risk of cultivation of GMB151 soybean on beneficial free-living nematodes was performed by characterizing the free-living nematode community from the rhizosphere and from between soybean rows after two years of growth of soybean. The trial included four genetically related soybean lines that differed in the presence or absence of native SCN resistance and of GMB151 resistance. Native resistance was represented by the conventionally bred *rgh1b* soybean cyst nematode resistance allele. The GMB151 trait was represented by Cry14Ab-1 expression. (BASF 2019, pp 189-196). Nematodes were extracted from soil near the root zone of the soybean plants and soil distant from the root zone between soybean rows. All nematodes were identified to the genus level and assigned to a functional trophic guild (predator, microbivore, fungivore, or omnivore). The nematode species assemblages were also used to score the soils on several ecological indices (Maturity Index, Enrichment Index, Structure Index, and Channel Index) which measure the disturbance, stability, complexity, and successional stage of the soil biotic community (Bongers 1990; Bongers and Bongers 1998; Ferris et al. 2001). This analysis showed that there was no difference between GMB151 and the non-modified comparator in terms of the trophic guilds represented by the free-living nematode community or any of the four ecological indices after two years of exposure to GMB 151 (BASF 2019). Thus, no effect of GMB 151 on the free-living nematode community or ecosystem services provided by that community due to the cultivation of GMB 151 is expected.

Risks to other non-target organisms

In addition to free-living soil nematodes, other non-target organisms may also be exposed to Cry14Ab-1 in GMB151 soybean plants or in soil residues. BASF presented data on the

exposure of non-target organisms (NTO) for environmental safety assessments and evaluations of potential adverse effects of the Cry protein. Test organisms were representative of pollinators, and soil-dwelling, predator, aquatic and avian organisms: Honey bee (*Apis mellifera*) adults and larvae; Collembola (*Folsomia candida*); Earthworm (*Eisenia fetida*); Ladybird beetles (*Colemegilla maculata* and *Coccinella setempunctata*); Green lacewing (*Chrysoperla carnea*); and Water flea (*Daphnia magna*) (BASF 2019 p.118-120). Cry14Ab-1 was also evaluated for acute toxicity to a mammal (mouse) and to one avian species Bobwhite quail (*Colinus virginianus*). No adverse effects were observed on the non-target species tested, and BASF concluded that Cry14Ab-1 is not likely to pose any risk to the tested NTOs at realistic field exposure levels (BASF 2019, p.187). BASF also conducted a thorough mammalian safety assessment for the Cry 14 Ab-1 expressed in GMB151 soybean and no adverse effects were observed.

In addition, Cry14Ab-1 was tested against several agriculturally relevant pests in standardized *in vitro* laboratory assays to evaluate its activity spectrum. Cry14Ab-1 was shown to be active against C. *elegans*, but no activity was detected against any of the assayed pests (BASF 2019, Table A3.1, p.159). The fact that no unexpected toxic activity of Cry14Ab-1 was found in the assays against plant pests strengthens the argument that unexpected activity of this protein against non-target organisms other than nematodes is unlikely.

Risks associated with HPPD-4

In addition to Cry14Ab-1, GMB151 soybean expresses a modified 4-hydroxyphenylpyruvate dioxygenase (HPPD-4). No adverse effect was found in a thorough mammalian safety assessment of HPPD-4 expressed in GMB151. The HPPD-4 protein is not expected to affect non-target organisms because HPPD proteins are ubiquitous and found in nearly all aerobic organisms, and amino acid sequences for the protein have been determined in bacteria, fungi, plants and animals including mammals. The HPPD-4 proteins have been characterized in organisms present in human food from plant, fungal and animal origin with good safety records, and therefore have a history of safe use (BASF 2019, p.37).

Conclusion

Therefore, based on the above analysis of data collected from assays to evaluate the activity spectrum of Cry 14Ab-1, effects on free living nematodes, and effects on other non-target organisms, APHIS concludes that exposure to and/or consumption of the modified plant and PIP are unlikely to have any adverse impacts to nontarget organisms beneficial to agriculture.

F. Potential for Enhanced Weediness of GMB151 soybean

APHIS assessed whether the GMB151 soybean is likely to become weedier (i.e. more prevalent, competitive, damaging or difficult-to-control in situations where it is not wanted) than the non-modified progenitor from which it was derived, or other varieties of

the crop currently under cultivation. The assessment considers the basic biology of the crop, the situations in which crop volunteers or feral populations are considered weeds, and an evaluation of GMB151 compared to the non-modified progenitor or the other reference varieties evaluated under field and laboratory conditions characteristic for the regions of the U.S. where GMB151 soybean is intended to be grown. The characteristics for the evaluation of GMB151 are related to establishment, competitiveness, reproduction, survival, persistence and/or spread that could influence weediness and the ability to manage the crop as a weed. For this crop, such characteristics include seed dormancy and germination, pollen fertility, agronomic and phenotypic traits, disease and pest susceptibility, abiotic stress tolerance, and plant-symbiont characteristics. The assessment also considers whether the engineered trait affects methods of control for the crop in situations where it is managed as a weed or volunteer in subsequent crops or in feral populations.

In the United States, soybean is not listed as a noxious weed species by the federal government (USDA-NRCS 2017) nor is listed as a weed in the major weed references (Holm et al. 1979; Randall 2017) . Soybean is not frost tolerant, does not survive freezing winter conditions (OECD 2000), and does not reproduce vegetatively. After crop harvest, soybean may germinate as a volunteer in the succeeding crop (OECD 2000). Mechanical methods or herbicides can be used to control volunteers. In managed ecosystems, soybean does not effectively compete with other cultivated plants or primary colonizers (OECD 2000).

BASF evaluated agronomic performance of GMB151 soybean by comparing it with the non-modified conventional counterpart Thorne, and reference varieties at eleven field sites across soybean growing regions in the United States, during the growing season of 2017. The data resulting from these field trials support the conclusion that GMB151 soybean lacks the potential to become weedy or to be a plant pest risk. Additionally, BASF also compared the seed germination potential under cold and warm germination tests of GMB151 to the non-modified counterpart Thorne, and demonstrated that there was no significant difference in germination potential between GMB151 and Thorne soybean seeds. (BASF 2019 pp. 115-116).

GMB151 soybean is resistant to HPPD-inhibitor herbicides, and remains sensitive to other widely used herbicides. As GMB151 is no different from cultivated soybean in any trait that might impact weediness, and remains sensitive to herbicides widely used for weed control in rotational crops of soybean, current practices to control volunteers will be effective. GMB151 also displays decreased susceptibility to SCN, which may allow the plants to better cope with other environmental stresses. There was no indication from agronomic assessments or from observations from field trials that this trait would otherwise affect interactions of GMB151 soybean with the abiotic environment (BASF 2019).

Based on the agronomic field data, environmental safety studies, and germination assays, for GMB151, as well as literature survey concerning weediness potential of the crop, GMB151 soybean is unlikely to persist as a troublesome weed or to have an impact on

current weed management practices. Furthermore, extensive post-harvest monitoring of field trial plots planted with the GMB151 soybean under USDA authorizations did not reveal any differences in survivability or persistence relative to other varieties of the same crop currently being grown. These data suggest that GMB151 soybean is no more likely to become a weed than conventional varieties of the crop. GMB151 soybean volunteers and feral populations can be managed using a variety of currently available methods and herbicides.

G. Potential Impacts on the Weediness of Any Other Plants with which GMB151 Soybean 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) Even in the existing floras, the occurrence of hybridization or introgression is reported to be widespread (Stace 1987; Rieseberg and Wendel 1993) (Preston et al. 2002).

It has been a common practice by plant breeders to artificially introgress traits from wild relatives into crop plants to develop new cultivars (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 (Ellstrand et al. 1999). This topic is covered in two sections: 1) the potential for gene flow, hybridization and introgression from the modified 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 the cultivated soybean (*G. max*) are well known and have previously been described. *Glycine max* and *G. soja* are predominantly self-pollinating with less than a 3% outcrossing rate because of the stringent cleistogamy of soybean flowers (Ahrent and Caviness 1994; OECD 2000; Ray et al. 2003). *Glycine max* and *G. soja* are sexually compatible and can hybridize resulting in fertile offspring, and two closely related interfertile wild relatives have been recognized: *G. gracilis* Skcortosov and *G. formosona* Hosok (Andersson and de Vicente 2010; Wang and Li 2011). Hybridization between species in the subgenus *Soja* with species in the subgenus *Glycine* has been achieved experimentally with difficulty and has resulted in sterile hybrids.

Glycine max is the only species of the genus *Glycine* grown in the United States, and no wild soybean species exist naturally in North America, but they are endemic in parts of Asia (OECD 2000; Wang and Li 2011). Some species in the subgenus *Soja* are occasionally grown in research plots, and there are no reports of escape and naturalization

of cultivated soybean plants in unmanaged habitats, or of other *Glycine* species in North America (OECD 2000).

Therefore, it is highly unlikely that gene flow and introgression will occur between GMB151 soybean and its wild relative species in the United States. APHIS has determined that any adverse consequences of gene flow from GMB151 soybean to wild or weedy species in the United States are highly unlikely.

Cultivated soybean plants are self-fertile and considered to be highly self-pollinating, pollination occurs before the flowers open. However, as much as 2.5% outcrossing may occur in some soybean cultivars when pollinators are present and other conditions are favorable (Ahrent and Caviness 1994). When soybean plants are grown directly adjacent to other soybean plants, the amount of natural cross pollination has generally been found to be 0.5 to1 percent (OECD 2000) although higher values (2.5 percent) occur in some varieties (Abud et al. 2007). Outcrossing can be reduced to 0 - 0.01 percent with a separation distance of 10 meters (Abud et al. 2007). At greater distances from the pollen source, cross pollination rates decrease rapidly.

Potential for enhanced weediness of recipients after gene flow and/or introgression

As discussed above in Section F "*Potential for Enhanced Weediness of GMB151 Soybean*", the expression of the integrated genetic materials in GMB151 soybean does not confer or enhance weedy characteristics of cultivated soybean other than enhancing herbicide resistance and reducing susceptibility to the soybean cyst nematode. Should gene flow and/or introgression from GMB151 soybean to its wild relatives occur, the introduced genetic materials are unlikely to cause enhanced weediness of recipient plants. Furthermore, cultivated soybean is the only soybean species grown in the U.S. and its territories and there are no sexually compatible wild relative species reported in natural environments in North America. Thus, USDA-APHIS has determined that any adverse consequences of gene flow and/or introgression from GMB151 soybean to wild relative or weedy species in the U.S. and its territories is highly unlikely.

Based on the information presented in the petition and in relevant literature, APHIS has reached the following conclusions. The genetic modification in the GMB151 soybean is not expected to increase the potential for gene flow, hybridization and/or introgression to sexually compatible taxa compared to the non-modified recipient or other varieties of commonly grown soybean. Gene flow, hybridization and/or introgression of genes from the GMB151 soybean to other sexually compatible relatives, including wild, weedy, feral or cultivated species in the United States and its territories is not likely to occur. Therefore, GMB151 soybean 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 the GMB151 soybean 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.

BASF evaluated the phenotypic and agronomic performance of GMB151 soybean in comparison to the non-modified Thorne and nine non-modified reference varieties representing the existing natural variability in soybeans. The agronomic assessment was performed during the 2017 growing season in 11 field trials in the U.S. The field trial sites and management systems used for the assessments represented geographically diverse regions, cropping practices, diverse soil types in areas of soybean production in the US. Growing and management conditions encompassed cultivation, irrigation, fertilizer and maintenance pest treatments were the same for all experimental plots (BASF 2019 Section 8 pp. 97-116).

No biologically relevant differences between GMB151 and the non-modified counterpart were observed on the agronomic assessment for early stand count, days to flowering, flowering duration, final stand count, days to maturity, fruit count, seed weight, and yield, crop development, and plant height (BASF 2019, p. 111 Table 39).

In a total of 132 observations for evaluation of responses to abiotic stressors, no differences were observed between GMB151 and the non-modified Thorne. The abiotic stressors observed were: cloudy/low light, drought, excess moisture in the soil, flooding, hail injury, heat stress, mineral toxicity, nutrient deficiency, soil compaction, soil crusting, sun scald, and wind damage (BASF 2019, p. 112, Table 41). Additionally, no differences between GMB151 and the conventional counterpart were found in 264 observations of biotic stressors that included arthropod pests (insects and spider mites) and diseases. (BASF 2019, p. 113 Table 42, p. 114 Table 43).

In general, management practices currently employed for conventional soybean cultivation are not expected to change if GMB151 soybean is determined to be no longer subject to the regulatory requirements of 7 CFR part 340 or to the plant pest provisions of the Plant Protection Act. BASF studies demonstrate that the cultivation practices needed for growing GMB151 soybean are essentially indistinguishable from practices used to grow conventional soybean (BASF 2019).

APHIS could not identify any significant changes to agricultural or cultivation practices (e.g. pesticide applications, tillage, irrigation, harvesting, etc.) from adoption of GMB151 soybean. When combined with commercially available soybean varieties that have native resistance to SCN, the durability of native and of GMB151 resistance are likely to be extended. It is likely that the introduction of GMB151 will reduce the negative impact of SCN infestation on soybean productivity, and no other 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 GMB151 Soybean Cannot Interbreed

APHIS examined the potential for the new genetic material inserted into GMB151 soybean 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 (HGT) between unrelated organisms is one of the most intensively studied fields in the biosciences since the late 1940s (Soucy et al. 2015), and the issue gained extra attention with the release of modified into the environment (Droge et al. 1998). Potential risks from stable HGT from genetically engineered organisms to another organism without reproduction or human intervention were reviewed by Keese (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 (Keese 2008; Soucy et al. 2015). HGT has been a major contributor to the spread of antibiotic resistance amongst bacteria; emergence of increased virulence in bacteria, eukaryotes and viruses; and, in the long run, to major transitions in evolution (Keese 2008).

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

Soybean GMB151 contains the following genes derived from bacteria: the coding sequence of a delta-endotoxin gene of *Bacillus thuringiensis*, and a modified coding sequence of a variant 4-hydroxyphenyl pyruvate dioxygenase gene (*hppdPf-4Pa*) of *Pseudomonas fluorescens* (BASF 2019 p.32).

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 (van den Eede et al. 2004; Keeling and Palmer 2008; 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 modified 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 (Keeling and Palmer 2008; Keese 2008; Isaza et al. 2011).

Second, in cases where review of sequence data implied that horizontal gene transfer occurred, these events are inferred to occur on an evolutionary time scale on the order of millions of years (Koonin et al. 2001; Brown 2003). Third, transgene DNA promoters and coding sequences are optimized for plant expression, not prokaryotic bacterial expression. Thus even if horizontal gene transfer occurred, proteins corresponding to the transgenes are not likely to be produced. Fourth, both the FDA (FDA 1998) and the European Food Safety Authority (EFSA 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 GMB151 soybean contains non-coding regulatory sequences from plant viruses: two copies of the *Cauliflower mosaic virus* (CaMV) 35S promoter region, the CaMV 35S terminator, and the leader sequence derived from *Tobacco etch virus* (TEV) (BASF 2019, p. 32). APHIS also considered whether horizontal transfer of DNA from GMB151 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 modified plants and infected related viruses are similar to recombinants found in mixed infections of the same viruses in non-modified plants, indicating that there was no novel recombination mechanism in the modified plants and no increased risk is expected over what is expected from mixed infections (Keese 2008; Turturo et al. 2008).

Non-homologous 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 (Morroni 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 contact 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, 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 (Yoshida et al. 2010). 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 2020). 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 and 24 –41% of mitochondrial (Xi et al. 2012) 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 GMB151 soybean, the DNA sequences were inserted into the nuclear genome, not the mitochondrial genome (BASF 2019).

If GMB151 soybean 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 GMB151 soybean. However, in both scenarios this newly introduced DNA would likely reside in somatic cells with little chance of reaching the germ cells, and 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 GMB151 soybean 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, and other relevant information to assess the plant pest risk of the GMB151 soybean

compared to the non-modified variety from which it was derived. APHIS concludes that GMB151 soybean is unlikely to pose a greater plant pest risk than the non-modified parental variety Thorne from which it was derived based on the following findings.

- No plant pest risk was identified from the transformation process or the insertion of new genetic material in the GMB151 soybean. The T-DNA sequences from *A. tumefaciens* inserted into GMB151 soybean lacked sequences from Tumor-inducing (Ti) plasmids normally responsible for the formation of galls in host plants. The left and right T-DNA border regions from the octopine synthase gene from *A. tumefaciens* present in GMB151 soybean and the sequences derived from *Cauliflower mosaic virus* and from *Tobacco etch virus* are non-coding sequences and do not cause disease.
- No increase in plant pest risk was identified in the GMB151 soybean from the expression of the inserted genetic material, of the new proteins Cry14Ab-1 and HPPD-4, or changes in metabolism or composition.
- Disease and pest incidence and/or damage were not observed to be significantly increased or atypical in the GMB151 soybean compared to the non-modified counterpart Thorne, or other comparators in field trials conducted in growing regions representative of where the GMB151 soybean is expected to be grown in the United States.
- Observed agronomic traits also did not reveal any significant differences that would indirectly indicate that the GMB151 soybean 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 the GMB151 soybean are unlikely to have any adverse impacts on organisms beneficial to agriculture based on the analysis of compositional, phenotypic and agronomic data. This data was supplemented by field observations of the impacts of GMB151 on the free-living soil nematode community.
- The GMB151 soybean is no more likely to become a weed or become weedier than conventional soybean varieties based on its observed agronomic characteristics, the weediness potential of soybean, and current management practices available to control soybean as a weed. Volunteers and feral populations of the GMB151 soybean resistant to HPPD-4 inhibitor herbicides can be managed using a variety of currently available methods and herbicides.
- The GMB151 soybean is not likely to increase the weed risk potential of other species with which it can interbreed in the United States or its territories. Gene flow, hybridization and/or introgression of inserted genes from the GMB151 soybean to other sexually compatible relatives with which it can interbreed is not likely to occur. GMB151 soybean does not confer or enhance weedy characteristics of cultivated soybean. Furthermore, there is no sexually compatible wild relative or weedy species of *Glycine* reported in natural environments in North America.
- Significant changes to agricultural or cultivation practices (e.g. pesticide applications, tillage, irrigation, harvesting, etc.) from adoption of the GMB151 soybean were not identified and therefore are not likely to increase plant diseases or pests or compromise their management.

- Horizontal gene transfer of the new genetic material inserted into the GMB151 soybean 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.

K. References

7 USC 7702. 2000. *Title IV Plant Protection Act*.

40 FR 260086. 2017. *HPPD-4 protein; exemption from the requirement of a tolerance*.

51 FR 23302. 1986. *Coordinated Framework for Regulation of Biotechnology*.

57 FR 22984. 1992. *Statement of Policy: Foods Derived from New Plant Varieties*.

80 FR 60414. 2015. *Clarifying Current Roles and Responsibilities Described in the Coordinated Framework for the Regulation of Biotechnology and Developing a Long-Term Strategy for the Regulation of the Products of Biotechnology*.

Abud S, de Souza P, Vianna G, Leonardecz E, Moreira C, Faleiro F, Junior J, Monteiro P, Rech E, and Aragao F. 2007. *Gene flow from transgenic to nontransgenic soybean plants in the Cerrado region of Brazil*. Genetics and Molecular Research 6, pp. 445-452.

Acuna R, Padilla BE, Florez-Ramos CP, Rubio JD, Herrera JC, Benavides P, Lee SJ, Yeats TH, Egan AN, Doyle JJ, and Rose JK. 2012. *Adaptive horizontal transfer of a bacterial gene to an invasive insect pest of coffee*. PNAS 109, pp. 4197-4202. Retrieved from http://www.pnas.org/content/early/2012/02/17/1121190109.abstract

Ahrent DK and Caviness CE. 1994. *Natural Cross-Pollination of Twelve Soybean Cultivars in Arkansas*. Crop Science 34, pp. 376-378. Retrieved from https://dl.sciencesocieties.org/publications/cs/pdfs/34/2/CS0340020376 Last accessed 09/22/2017.

Allison R, Dougherty W, Parks T, Willis L, Johnston RE, Kelly M, and FB. A. 1985. *Biochemical analysis of the capsid protein gene and capsid protein of tobacco etch virus:n'terminal amino acids are located on the virion surface*. Virology, pp. 309-316.

Andersson M and de Vicente M. 2010. Soybean. In: *Gene flow between crops and their wild relatives* (Johns Hopkins University Press).

Bandara A, Weerasooriya D, ., Bradley C, Allen T, ., and Esker D. 2020. *Dissecting the economic impact of soybean diseases in the United States over two decades*. PLoS ONE 15, pp. e0231141.

Barr CM, Neiman M, and Taylor DR. 2005. *Inheritance and recombination of mitochondrial genomes in plants, fungi and animals*. New Phytologist 168, pp. 39-50; DOI: 10.1111/j.1469-8137.2005.01492.x. Retrieved from http://onlinelibrary.wiley.com/doi/10.1111/j.1469-8137.2005.01492.x/pdf Last accessed Aug 2014.

BASF. 2019. *Petition for a Determination of Nonregulated Status for Plant-Parasitic Nematode-Protected and Herbicide Tolerant GMB151 Soybean*.

Bongers T. 1990. *The maturity index: an ecological measure of environmental disturbance based on nematode species composition*. Oecologia 83, pp. 14-19.

Bongers T and Bongers M. 1998. *Functional diversity of nematodes*. Applied Soil Ecology 10, pp. 239-251.

Bravo A, Gomez I, Porta H, Garcia-Gomez BI, Rodriguez-Almazan C, Pardo L, and Soberon M. 2012. *Evolution of Bacillus thuringiensis Cry toxins insecticidal activity*. Microb Biotechnol 6, pp. 17-26. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/22463726

Brown JR. 2003. *Ancient horizontal gene transfer*. Nature Reviews Genetics 4, pp. 121-132; doi:110.1038/nrg1000. Retrieved from http://www.nature.com/nrg/journal/v4/n2/full/nrg1000.html Last accessed Aug 2014.

Chung and Singh R. 2008. *Broadening the Genetic Base of Soybean: A Multidisciplinary Approach*. Critical Reviews in Plant Sciences 27, pp. 295-341.

Droge M, Puhler A, and Selbitschka W. 1998. *Horizontal gene transfer as a biosafety issue: A natural phenomenon of public concern*. Journal of Biotechnology 64, pp. 75-90.

EFSA. 2009. *Consolidated presentation of the joint Scientific Opinion of the GMO and BIOHAZ Panels on the "Use of Antibiotic Resistance Genes as Marker Genes in Genetically Modified Plants" and the Scientific Opinion of the GMO Panel on "Consequences of the Opinion on the Use of Antibiotic Resistance Genes as Marker Genes in Genetically Modified Plants on Previous EFSA Assessments of Individual GM Plants*. The European Food Safety Authority Journal 7, pp. 1-107. Retrieved from http://www.efsa.europa.eu/en/efsajournal/doc/1108.pdf Last accessed Aug 2014.

Ellstrand NC, Prentice HC, and Hancock JF. 1999. *Gene flow and introgression from domesticated plants into their wild relatives*. Annual Review of Ecology and Systematics 30, pp. 539-563; DOI: 510.1146/annurev.ecolsys.1130.1141.1539. Retrieved from http://www.annualreviews.org/doi/abs/10.1146%2Fannurev.ecolsys.30.1.539 Last accessed Aug 2014.

EPA-FIFRA-SAP. 2006. *Plant Incorporated Protectants Based on Virus Coat Protein Genes: Science Issues Associated with the Proposed Rule*. Retrieved from https://archive.epa.gov/scipoly/sap/meetings/web/pdf/minutes1205.pdf Last accessed 09/28/2017.

FDA. 1998. *Use of antibiotic resistance marker genes in transgenic plants*. Retrieved from https://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/Biotechnology/ucm096135.htm

Ferris H, Bongers T, and de Goede RGM. 2001. *A framework for soil food web diagnostics: extension of the nematode faunal analysis concept*. Applied Soil Ecology 18, pp. 13-29.

Frankenhuyzen K. 2009. *Insecticidal activity of Bacillus thuringiensis crystal proteins*. Journal of Invertebrate Pathology 101, pp. 1-16.

Frischmuth T and Stanley J. 1998. *Recombination between viral DNA and the transgenic coat protein gene of African cassava mosaic geminivirus*. Journal of General Virology 79, pp. 1265-1271. Retrieved from

http://vir.sgmjournals.org/content/79/5/1265.full.pdf+html Last accessed Aug 2014.

Fritze IM, Linden L, Freigang J, Auerbach G, Huber R, and Steinbacher S. 2004. *The Crystal Structures of Zea mays and Arabidopsis 4-Hydroxyphenylpyruvate Dioxygenase*. Plant Physiology 134, pp. 1388-1400.

Fuchs M and Gonsalves D. 2007. *Safety of virus-resistant transgenic plants two decades after their introduction: Lessons from realistic field risk assessment studies*. Annual Review of Phytopathology 45:, pp. 173-202; doi:110.1146/annurev.phyto.1145.062806.094434. Retrieved from http://www.annualreviews.org/doi/full/10.1146/annurev.phyto.45.062806.094434?url_ver=Z39.88-2003 Last accessed Aug 2014.

Grant V. 1981. *Plant Speciation, 2nd Edition*. New York: Columbia University Press, 563 pp.

Grefen C, Donald N, Hashimoto K, Kudla J, Schumacher K, and Blatt MR. 2010. *A ubiquitin-10 promoter-based vector set for fluorescent protein tagging facilitates temporal stability and native protein distribution in transient and stable expression studies*. The Plant Journal 64, pp. 355-365.

Hartman G, Pawlowski M, Herman T, and Eastburn D. 2016. *Organically Grown Soybean Production in the USA: Constraints and Management of Pathogens and Insect Pests*. Agronomy 6, pp. 16.

Hartman GL, West ED, and Herman TK. 2011. *Crops that feed the World 2. Soybean—worldwide production, use, and constraints caused by pathogens and pests*. Food Security 3, pp. 5-17.

Hegde SG, Nason JD, Clegg JM, and Ellstrand NC. 2006. *The evolution of California's wild radish has resulted in the extinction of its progenitors*. Evolution 60, pp. 1187-1197. Retrieved from http://dx.doi.org/10.1554/05-634.1 Last accessed Dec 2014.

Holm L, Pancho JV, Herberger JP, and Plucknett DL. 1979. *A Geographical Atlas of World Weeds*.

Hymowitz T. 1970. *On the domestication of the soybean*. Economic Botany 24, pp. 408-421. Retrieved from https://doi.org/10.1007/BF02860745

Hymowitz T and Newell CA. 1981. *Taxonomy of the Genus Glycine, Domestication and Uses of Soybeans*. Economic Botany 35, pp. 272-288. Retrieved from http://www.jstor.org/stable/4254296

Hymowitz T and Harlan JR. 1983. *Introduction of Soybean to North America by Samuel Bowen in 1765*. Economic Botany 37, pp. 371-379. Retrieved from http://www.jstor.com/stable/425429

Isaza L, Opelt K, Wagner T, Mattes E, Bieber E, Hatley E, Roth G, Sanjuán J, Fischer H, Sandermann H, Hartmann A, and D. E. 2011. *Lack of glyphosate resistance gene transfer from Roundup Ready ® soybean to Bradyrhizobium japonicum under field*. Last accessed 9/26/2017.

Kaneko T, Nakamura Y, Sato S, Minamisawa K, Uchiumi T, Sasamoto S, Watanabe A, Idesawa K, Iriguchi M, Kawashima K, Kohara M, Matsumoto M, Shimpo S, Tsuruoka H, Wada T, Yamada M, and Tabata S. 2002. *Complete Genomic Sequence of Nitrogen-fixing Symbiotic Bacterium Bradyrhizobium japonicum USDA110*. DNA Research 9, pp. 189 - 197.

Kay R, Chan A, Daly M, and McPerson J. 1987. *Duplication of CaMV 35S promoter sequences creats a strong enhancer for plant genes*. Science 236, pp. 1299-1302.

Keeling PJ and Palmer JD. 2008. *Horizontal gene transfer in eukaryotic evolution*. Nature Reviews Genetics 9, pp. 605-618; doi:610.1038/nrg2386. Retrieved from http://www.nature.com/nrg/journal/v9/n8/full/nrg2386.html Last accessed Aug 2014.

Keese P. 2008. *Risks from GMOs due to horizontal gene transfer*. Environmental Biosafety Research 7, pp. 123-149; DOI: 110.1051/ebr:2008014. Retrieved from http://journals.cambridge.org/action/displayAbstract?fromPage=online&aid=8208895&fileId=S1635792208000146 Last accessed Dec 2014.

Khoury CK, Greene S, Wiersema J, Maxted N, Jarvis A, and Struik PC. 2013. *An inventory of crop wild relatives of the United States*. Crop Science 53, pp. 1496; doi:1410.2135/cropsci2012.1410.0585. Retrieved from https://www.crops.org/publications/cs/abstracts/53/4/1496 Last accessed Aug 2014.

Koonin EV, Makarova KS, and Aravind L. 2001. *Horizonal gene transfer in prokaryotes: Quantification and classification*. Annual Review of Microbiology 55:, pp. 709-742. Retrieved from http://www.annualreviews.org/doi/full/10.1146/annurev.micro.55.1.709?url_ver=Z39.88-2003 Last accessed Aug 2014.

Lebrun M, Leroux B, and Saillan A. 1996. US Patent.

Morroni M, Jacquemond M, and Tepfer M. 2013. *Deep sequencing of recombinant virus populations in transgenic and nontransgenic plants infected with Cucumber mosaic virus*. Molecular Plant-Microbe Interactions 26, pp. 801-811. Retrieved from http://dx.doi.org/10.1094/MPMI-02-13-0057-R Last accessed Aug 2014.

Munger P, Bleiholder H, Hack H, Hess M, Stauss R, van den Boom T, and Weber E. 1997. *Phenological growth stages of the soybean plant (Glycine max L. Merr.): codification and description according to the BBCH scale.* J. Agronomy and Crop Science, pp. 219-217.

Niblack T, Lambert K, and Tylka G. 2006. *A Model Plant Pathogen from the Kingdom Animalia: Heterodora glycines the Soybeant Cyst Nematode*. Annu. Rev. Phytopathol. 44, pp. 283-303.

OECD. 1997. *Consensus Document of Information used in the Assessment of Environmental Applications Involving Pseudomonas*.

OECD. 2000. *Consensus Document on the Biology of Glycine max (L.) Merr. (Soybean) ENV/JM/MONO(2000)9*. Retrieved from http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?doclanguage=en&cote=env/jm/mono(2000)9 Last accessed Aug 2020.

OEPP-EPPO. 2018. *PM 7/89 (2) Heterodera glycines*. EPPO Bulletin 48, pp. 64-77.

Poree F, Volker H, Lange G, Laber B, Peters C, and Schouten L. 2014. US Patent.

Preston CD, Pearman DA, and Dines TD. 2002. *New Atlas of the British flora*. Oxford University Press.

Randall RP. 2017. *A Global Compendium of Weeds. Third Edition.* Perth, Western Australia. Retrieved from https://www.researchgate.net/profile/Roderick_Randall

Ray JD, Kilen TC, Abel CA, and Paris RL. 2003. *Soybean natural cross-pollination rates under field conditions*. Environ. Biosafety Res. 2, pp. 133-138. Retrieved from https://doi.org/10.1051/ebr:2003005

Reyes-Ramirez A and Ibarra JE. 2008. *Plasmid patterns of Bacillus thuringiensis type strains*. Appl Environ Microbiol 74, pp. 125-129. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/18024687

Richards TA, Soanes DM, Foster PG, Leonard G, Thornton CR, and Talbot NJ. 2009. *Phylogenomic analysis demonstrates a pattern of rare and ancient horizontal gene transfer between plants and fungi*. Plant Cell 21, pp. 1897-1911. Retrieved from http://www.plantcell.org/content/21/7/1897.long Last accessed Aug 2014.

Richardson AO and Palmer JD. 2007. *Horizontal gene transfer in plants*. Journal of Experimental Botany 58, pp. 1-9; doi:10.1093/jxb/erl1148. Retrieved from http://jxb.oxfordjournals.org/content/58/1/1.full.pdf+html Last accessed Aug 2014.

Rieseberg L and Wendel J. 1993. *Introgression and Its Consequences in Plants*. Hybrid Zones and the Evolutionary Process, pp. 70-109.

Ruan L, Crickmore N, Peng D, and Sun M. 2015. *Are nematodes a missing link in the confounded ecology of the entomopathogen Bacillus thuringiensis?* Trends in Microbiology 23, pp. 341-346.

Sanahuja G, Banakar R, Twyman RM, Capell T, and Christou P. 2011. *Bacillus thuringiensis: a century of research, development and commercial applications*. Plant Biotechnol J 9, pp. 283-300. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/21375687

Sanfacon H, Brodman P, and Hohn T. 1990. *A dissection of the cauliflower mosaic virus polyadenylation signal*. Genes and Development, pp. 141-149.

Sedivy EJ, Wu F, and Hanzawa Y. 2017. *Soybean domestication: the origin, genetic architecture and molecular bases*. The New phytologist 214, pp. 539-553. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/28134435

Sherman-Broyles S, Bombarely A, Powell AF, Doyle JL, Egan AN, Coate JE, and Doyle JJ. 2014. *The wild side of a major crop: soybean's perennial cousins from Down Under*. American journal of botany 101, pp. 1651-1665. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/25326613

Smith D, Chilvers M, Dorrance A, Hughes T, Mueller D, Niblack T, and Wise K. 2016. *Soybean Disease Management*. Crop Protection Network.

Soltis DE, Soltis PS, and Rieseberg LH. 1993. *Molecular data and the dynamic nature of polyploidy*. Critical Reviews in Plant Sciences 12, pp. 243-273; 210.1080/07352689309701903. Retrieved from http://www.tandfonline.com/doi/abs/10.1080/07352689309701903#.UhekuRtwq-w Last accessed Dec 2014.

Soucy SM, Huang J, and Gogarten JP. 2015. *Horizontal gene transfer: building the web of life*. Nature reviews. Genetics 16, pp. 472-482. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/26184597

Stace CA. 1987. *Hybridization and the plant species*. Differentiation Patterns in Higher Plants, pp. 115–127.

Thompson JR and Tepfer M. 2010. *Assessment of the benefits and risks for engineered virus resistance*. Retrieved from

http://www.sciencedirect.com/science/article/pii/S0065352710760024 Last accessed Dec 2014.

Turturo C, Friscina A, Gaubert S, Jacquemond M, Thompson JR, and Tepfer M. 2008. *Evaluation of potential risks associated with recombination in transgenic plants expressing viral sequences*. Journal of General Virology 89:, pp. 327-335; DOI 310.1099/vir.1090.83339-83330. Retrieved from http://vir.sgmjournals.org/content/89/1/327.full.pdf+html Last accessed Aug 2014.

Tylka G and Marett C. 2014. *Distribution of the Soybean Cyst Nematode, Heterodera glycines, in the United States and Canada: 1954 to 2014*. Plant Health Progress.

USDA-APHIS. 2017. *List of Deregulated GE crops*. Retrieved from https://www.aphis.usda.gov/aphis/ourfocus/biotechnology/permits-notifications-petitions/petitions/petition-status

USDA-APHIS. 2020. *Plant Pests and Diseases Programs* Retrieved from https://www.aphis.usda.gov/aphis/ourfocus/planthealth/plant-pest-and-disease-programs/pests-and-diseases/plant-disease Last accessed June 16, 2020.

USDA-FAS. 2017. *World Agricultural Production*. Retrieved from http://usda.mannlib.cornell.edu/usda/current/worldag-production/worldag-production-10-12-2017.pdf

USDA-NASS. 2019. *Soybean Acres United States 2019.pdf*. Retrieved from https://www.nass.usda.gov/Charts_and_Maps/graphics/soyac.pdf Last accessed March 21 2020.

USDA-NASS. 2020. *Soybeans 2019 Planted Acres by County for Selected States*. Retrieved from https://www.nass.usda.gov/Charts_and_Maps/graphics/SB-PL-RGBChor.pdf

USDA-NRCS. 2017. *Plant Database - Soybean*. Retrieved from https://plants.usda.gov/core/profile?symbol=GLMA4

USDA-NRCS. 2020. *Striga asiatica (L.) Kuntze, Asiatic witchweed*. National Plant Data Center. Retrieved from http://plants.usda.gov/core/profile?symbol=STAS2 Last accessed 21 August 2013.

USDA FDA EPA. 2017. *Modernizing the Regulatory System for Biotechnology Products: Final Version of the 2017 Update to the Coordinated Framework for the Regulation of Biotechnology*.

van den Eede G, Aarts H, Buhk HJ, Corthier G, Flint HJ, Hammes W, Jacobsen B, Midtvedt T, van der Vossen J, von Wright A, Wackernagel W, and Wilcks A. 2004. *The relevance of gene transfer to the safety of food and feed derived from genetically modified (GM) plants*. Food Chem Toxicol 42, pp. 1127-1156. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/15123384

Wang KJ and Li XH. 2011. *Interspecific gene flow and the origin of semi-wild soybean revealed by capturing the natural occurrence of introgression between wild and cultivated soybean populations*. Plant Breeding 130, pp. 117-127.

Wei JZ, Hale K, Carta L, Platzer E, Wong C, Fang SC, and Aroian RV. 2003. *Bacillus thuringiensis crystal proteins that target nematodes*. Proc Natl Acad Sci U S A 100, pp. 2760-2765. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/12598644

Westphal A, Abney T, Xing L, and Shaner G. 2018. *Sudden death syndrome of soybean*. Retrieved from https://www.apsnet.org/edcenter/disanpath/fungalasco/pdlessons/Pages/SuddenDeath.aspx Last accessed July 6, 2020.

Wood DW, Setubal JC, Kaul R, Monks DE, Kitajima JP, Okura VK, Zhou Y, Chen L, Wood GE, Almeida NF, Woo L, Chen YC, Paulsen IT, Eisen JA, Karp PD, Bovee D, Chapman P, Clendenning J, Deatherage G, Gillet W, Grant C, Kutyavin T, Levy R, Li MJ, McClelland E, Palmieri A, Raymond C, Rouse G, Saenphimmachak C, Wu ZN, Romero P, Gordon D, Zhang SP, Yoo HY, Tao YM, Biddle P, Jung M, Krespan W, Perry M, Gordon-Kamm B, Liao L, Kim S, Hendrick C, Zhao ZY, Dolan M, Chumley F, Tingey SV, Tomb JF, Gordon MP, Olson MV, and Nester EW. 2001. *The genome of the natural genetic engineer Agrobacterium tumefaciens C58*. Science 294, pp. 2317-2323. Retrieved from ISI:000172817200037

WSSA. 1998. *"Herbicide Resistance" and "Herbicide Tolerance" defined. (Technology Note)*. Weed Technology 12, pp. 789. Retrieved from http://www.jstor.org/stable/3989101 Last accessed 04/09/2013.

Xi ZX, Bradley RK, Wurdack KJ, Wong KM, Sugumaran M, Bomblies K, Rest JS, and Davis CC. 2012. *Horizontal transfer of expressed genes in a parasitic flowering plant*. BMC Genomics 13, pp. 227. Retrieved from http://www.biomedcentral.com/1471-2164/13/227

Yoshida S, Maruyama S, Nozaki H, and Shirasu K. 2010. *Horizontal gene transfer by the parasitic plant Striga hermonthica*. Science 328, pp. 1128; DOI:1110.1126/science.1187145. Retrieved from http://www.sciencemag.org/content/328/5982/1128.full Last accessed September 26, 2017.

Zhu B, Lou MM, Xie GL, Zhang GQ, Zhou XP, Li B, and Jin GL. 2011. *Horizontal gene transfer in silkworm, Bombyx mori*. BMC Genomics 12, pp. 248.