Syngenta and Bayer Petition (12-215-01p) for Determination of Non-regulated Status of SYHT0H2 Soybean

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Plant Pest Risk Assessment

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A. Introduction

Syngenta Seeds, Inc., (Syngenta) and Bayer CropScience AG (Bayer) have petitioned the United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS) for a determination that Event SYHT0H2 double herbicide-tolerant[1] soybean (hereafter referred to as SYHT0H2 soybeans) is unlikely to pose a plant pest risk and, therefore, should no longer be a regulated article under APHIS' 7 Code of Federal Regulations (CFR) part 340. This petition was assigned the number 12-125-01p, and is hereafter referenced as Syngenta and Bayer 2012. APHIS administers 7 CFR part 340 under the authority of the plant pest provisions of the Plant Protection Act (PPA) of 2000 (7 U.S.C. 7701 *et seq*.)[2]. This plant pest risk assessment was conducted to determine if SYHT0H2 soy is unlikely to pose a plant pest risk.

APHIS regulations in 7 CFR part 340 regulate the introduction (importation, interstate movement, or release into the environment) of certain GE organisms and products. A GE organism is no longer subject to the plant pest provisions of the PPA or to the regulatory requirements of Part 340 when APHIS determines that it is unlikely to pose a plant pest risk. A GE organism is considered a regulated article under part 340 if the donor organism, recipient organism, or vector, or vector agent used in engineering the organism belongs to any genera or taxa designated in 7 CFR 340.2 and meets the definition of plant pest, or is an unclassified organism and/or an organism whose classification is unknown, or any product which contains such an organism, or any other organism or product altered or produced through genetic engineering which the APHIS Administrator determines is a plant pest or has reason to believe is a plant pest[3].

The Event SYHT0H2 soybean (*Glycine max* cultivar Jack) expresses proteins which impart herbicide tolerance, AvHPPD-03 from oat (*Avena sativa*) and phosphinothricin acetyltransferase (PAT) from *Streptomyces viridochromogenes*. The AvHPPD-03 and PAT proteins expressed in the soybean protect the plant from the application of the HPPD-inhibiting and glufosinate-amonium herbicides, respectively. SYHT0H2 soybean

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[1] Syngenta and Bayer have described the phenotype of SYHT0H2 soybeans as "herbicide tolerant" and historically APHIS has also referred to GE 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 SYHT0H2 soybeans has an "inherited ability to survive and reproduce following exposure to a dose of herbicide normally lethal to the wild type". By the WSSA, (1998) 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] Plant Protection Act in 7 U.S.C. 7702 § 403(14) defines plant pest as: "Plant Pest - The term "plant pest" means any living stage of any of the following that can directly or indirectly injure, cause damage to, or cause disease in any plant or plant product: (A) A protozoan. (B) A nonhuman animal. (C) A parasitic plant. (D) A bacterium. (E) A fungus. (F) A virus or viroid. (G) An infectious agent or other pathogen. (H) Any article similar to or allied with any of the articles specified in the preceding subparagraphs."

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

was produced by the transformation of immature soybean seed using disarmed *Agrobacterium tumefaciens*. Because five of the regulatory sequences used to facilitate expression of the herbicide tolerance genes in SYHT0H2 soybean (figwort mosaic virus (FMV), cauliflower mosaic virus, Cestrum yellow leaf curling virus, tobacco mosaic virus (Syngenta and Bayer, 2012, p. 18), were derived from plant pests and *Agrobacterium tumefaciens* used for transformation is a plant pest, SYHT0H2 soybean has been considered a regulated article under APHIS regulations at 7 CFR part 340.

Potential impacts in this Plant Pest Risk Assessment are those that pertain to plant pest risk associated with SYHT0H2 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 SYHT0H2 soybean is unlikely to pose a plant pest risk. APHIS regulations in 7 CFR 340.6(c) specify the information needed for consideration in a petition for nonregulated status. APHIS will assess information submitted by the applicant about SYHT0H2 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 regulated article on nontarget organisms; weediness of the regulated article; impact on the weediness of any other plant with which it can interbreed; changes to agricultural or cultivation practices that may impact diseases and pests of plants; and transfer of genetic information to organisms with which it cannot interbreed.

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

EPA regulates under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) (7 U.S.C. 136 *et seq.)* the distribution, sale, use and testing of pesticidal substances produced in plants and microbes, including those pesticides that are produced by an organism through techniques of modern biotechnology. EPA also sets tolerance limits for residues of pesticides on and in food and animal feed, or establishes an exemption from the requirement for a tolerance, under the Federal Food, Drug and Cosmetic Act (FFDCA) (21 U.S.C. Chapter 9). Prior to registration for a new use for a new or previously registered pesticide, EPA must determine through testing that the pesticide does not cause unreasonable adverse effects on humans, the environment, and nontarget species when used in accordance with label instructions. EPA must also approve the language used on the pesticide label in accordance with 40 CFR part 158. Other applicable EPA regulations include 40 CFR part 152 - Pesticide Registration and Classification Procedures, part 174 - Procedures and Requirements for Plant Incorporated Protectants (PIPs) and part 172 - Experimental Use Permits.

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 (FDA 2006) and a more comprehensive voluntary consultation process prior to commercial distribution of food or feed (57 FR 22984).

Syngenta and Bayer have made a request to the U.S. Environmental Protection Agency to amend the use of mesotrione on SYHT0H2 soybeans. Currently, EPA allows a maximum season rate application of 1,333 g a.i./ha of mesotrione in corn (Syngenta and Bayer, 2012, p. 133). These companies are also pursuing the consultation process with the Food and Drug Administration (FDA) concerning the regulation of products derived from new plant varieties, including those of biotechnology, as well as with the regulatory offices of 12 countries, the Russian Federation and the European Union (Syngenta and Bayer, 2012, p. 19).

B. Development of SYHT0H2 soybean

There are no genetically engineered events for the insertion of the *hppd* gene into soybean or any other crop species to date, or soybean tolerant to glufosinate, although two petitions (09-349-01p, 11-234-01p) that confer tolerance to those herbicides are currently in the review process by APHIS Biotechnology Regulatory Services (BRS). The insertion of the *hppd* gene derived from oats (*Avena sativa*), confers to SYHT0H2 soybeans tolerance to the 4-hydroxyphenyl-pyruvate (HPPD) inhibitor herbicide mesotrione (Mitchell, 2001). The inhibition of the 4-HPPD enzyme disrupts the metabolism of the amino acid tyrosine (Wu, 2002). The insertion of the *pat* gene from the bacterium *Streptomyces viridochromogenes* produces the enzyme phosphinothricin acetyltransferase (PAT) that inactivates the herbicide gufosinate-ammonium, allowing SYHT0H2 soybeans to tolerate contact with both herbicides. Syngenta and Bayer have developed SYHT0H2 soybean to be tolerant to both herbicides.

HPPD-inhibitor herbicide controls a broad spectrum of grass and broadleaf weeds and is effective on difficult to control weeds (Mitchell, 2001; Johnson, 2002; Bollman, 2008). The combined effects of desorption (release of the compound from the soil) and degradation resupply the soil solution with a bioactive product. Because of its adsorption characteristics, the application rate of isoxaflutole is adjusted for soil texture and organic matter. Depending on the concentration of organic matter, the half-life of HPPD in soil can be up to 30 days (Inoue, 2009). Due to the potential for leaching, especially on permeable soils, isoxaflutole is a restricted-use pesticide. The label prohibits its use on sandy soils with less than 2% organic matter where the water table is less than 25 feet from the surface. Isoxaflutole has a bleaching effect as chlorophyll is broken down in sunlight but is not replaced. The symptoms first appear on leaf edges and tips as this is the site of new carotenoid synthesis (Johnson, 2002). Once in the plant, isoxaflutole is rapidly converted into diketonitrile (DKN) which works by inhibiting the production of 4-hydroxyphenyl-pyruvate dioxygenase (HPPD). Inhibition of HPPD stops the biosynthesis of plastoquinone, a carotenoid pigment that is needed for electron transport process of photosynthesis (http://www.uniprot.org/keywords/618).

Glufosinate ammonium's salt, the herbicidal product, irreversibly inhibits glutamine synthetase in plants, blocking the synthesis of the amino acid glutamine from glutamate and ammonia. This results in the rapid accumulation of potentially toxic levels of ammonia in the cell, a by-product of photosynthesis (Shaner, 2003). It also results in a deficiency in the production of several amino acids, an inhibition of photosynthesis and finally the death of the plant cell (Müller, 2001). Glufosinate, an essential amino acid in animals and plants, is initially transformed to the corresponding oxo acid, 2-oxo-4-(hydroxymethylphosphinyl) butanoic acid (PPO), possibly by a transamination reaction. The intermediate PPO is decarboxylated to the main metabolite 3 (hydroxymethyl-phosphinyl) propionic acid which is considered stable in plants (Müller, 2001; Shaner, 2003).

In the U.S. soybeans are grown on over 78.9 million acres (Figure 1) with a value of $31.7 billion in 2009 (USDA-ERS, 2011). Growers select soybean lines adapted to the different environmental and climatic features, weed and disease pressures, cost of seed and other inputs, technology fees, human safety, ease and flexibility of the production system and marketing reasons (Reddy, 2001; Gianessi, 2006).

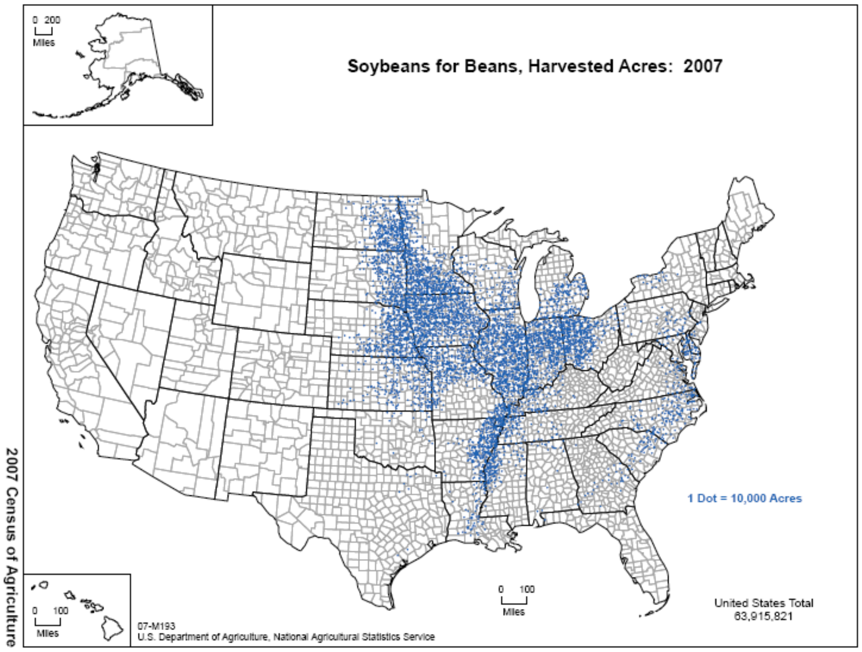

Figure 1. Soybean production areas in the U.S. (USDA-NASS, 2011).

For the past approximately 28 years soybean yields in the U.S. have increased 65% at an annual rate of about 2.3% (Figure 2, next page). In order to meet the domestic and export demands, soybean productivity in the U.S. has been accomplished by both increasing the area under cultivation and by these constant yield increases per unit area.

For example, from 1924 to 2010, soybean acreage increased almost 50-fold, but in the last decade the increase in planted area had been only 6% (USDA-NASS, 2011). The annual improvement in U.S. soybean yield can be attributed to genetic and agronomic innovations and better control of weeds, pests and diseases that provide producers better tools to meet production demands (Specht, 1999), depending also on continuing infusions of genetic resources for yield stability and growth (USDA-ERS, 2011).

One of the agronomic innovations in soybeans has been the development of varieties that are tolerant to herbicides. From the early adoption of glyphosate-resistant soybean in 1996, a great adoption increment has followed, from approximately 5% adoption in 1997 to over 25% in 2006 (Dill, 2007). Because major crops such as soybeans, cotton and corn have been genetically engineered to tolerate glyphosate herbicide, dozens of weed species around the world have shown resistance to this herbicide (Powles, 2008). To maintain the high productivity of soybeans it is necessary to have adequate tools for the control of weeds. SYHT0H2 soybeans tolerate the contact with two herbicides that are different in their mode-of-action to glyphosate. SYHT0H2 soybeans have been tested in the U.S. since 2008 in 24 states under 20 different APHIS-BRS notifications (Syngenta and Bayer, 2012, appendix A, p. 137).

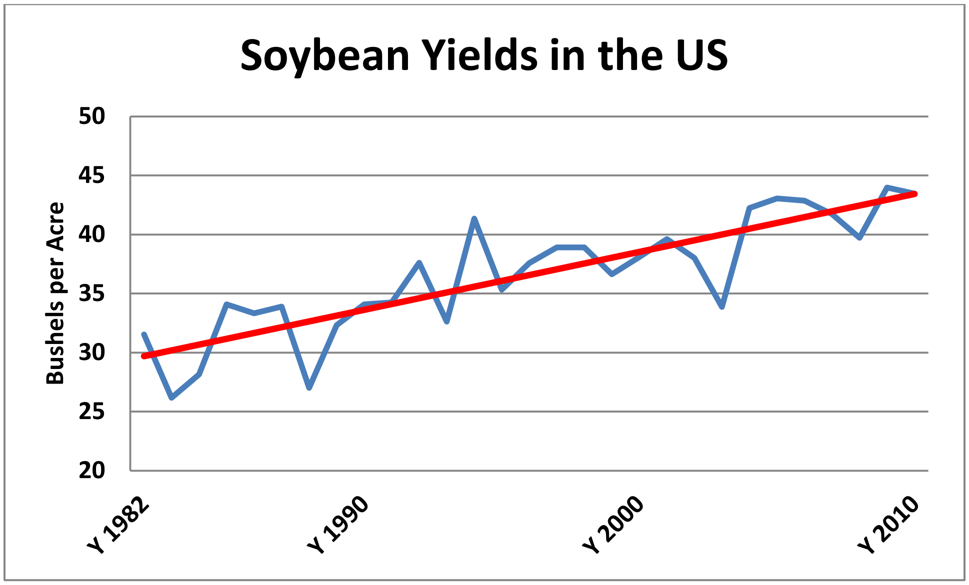

Figure 2. Soybean yield trend in the U.S. (USDA ERS 2011).

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

To inform the potential hazards resulting from the genetic modification and potential routes of exposure related to the inserted DNA and its expression products, APHIS assessed data and information presented in the petition related to: the transformation process; the source of the inserted genetic material and its function in both the donor organism and SYHT0H2 soybeans; 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 SYHT0H2 soybeans relative to the nontransgenic counterparts. The assessment encompasses a consideration of the expressed proteins and any observed or anticipated effects on plant metabolism including, any relevant changes in levels of metabolites, antinutrients, or nutrients in harvested seed and fiber derived from SYHT0H2 soybeans event compared to those in the conventional counterpart or to other comparators.

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 SYHT0H2 soybeans; or for expression of inserted DNA, new proteins or enzymes, or changes in metabolism to affect plant pest or diseases, nontarget beneficial organisms, weediness, agricultural practices that impact pest or diseases or their management, or plant pest risks through horizontal gene flow.

Description of the genetic modification and inheritance of inserted DNA

The number of T-DNA integration sites, number of copies of the functional elements of the transformation plasmid pSYN15954, and the presence / absence of plasmid backbone sequence in the SYHT0H2 soybean genome was determined by Southern blot analyses (Syngenta and Bayer, 2012, figures V-1 and V-2, table V-1, p. 35-38). Herbicide tolerant Event SYHT0H2 soybean contains, at a single locus within the soybean genome that is stably inherited, a single copy of *avhppd-03,* four copies of *pat,* a single copy of the avhppd-03 enhancer complex sequence, two copies of the CaMV 35S promoter, two copies of the CMP promoter, two copies of the TMV enhancer, and five copies of the NOS terminator. It does not contain any extraneous DNA fragments of these functional elements elsewhere in the SYHT0H2 soybean genome, and it does not contain the FMV enhancer or plasmid backbone sequence from pSYN15954 (Syngenta and Bayer, 2012, p. 38-43).

The transformation plasmid pSYN15954 was used to produce SYHT0H2 soybeans by *Agrobacterium tumefaciens*–mediated transformation of immature “Jack” soybean seed. The DNA region between the left and right borders of the transformation plasmid included gene-expression cassettes for avhppd-03, pat-03-01, and pat-03-02. The avhppd-03 expression cassette consisted of the avhppd-03 coding region regulated by a synthetic minimal plant (SMP) promoter, figwort mosaic virus (FMV) enhancer, CaMV 35S enhancer (35S enhancer), tobacco mosaic virus (TMV) enhancer, and nopaline synthase (NOS) polyadenylation terminator sequence. The pat-03-01 expression cassette consisted of the pat-03-01 coding region regulated by a CaMV 35S promoter (35S promoter) and NOS terminator sequence. The pat-03-02 expression cassette consisted of the pat-03-02 coding region regulated by a Cestrum yellow leaf curling virus promoter (CMP), TMV enhancer, and NOS terminator sequence.

Genetic element	Description
avhppd-03 cassette	
FMV enhancer	Figwort mosaic virus transcriptional enhancer region (similar to Accession No. X06166.1 (NCBI-Protein, 2012)), which increases gene expression (Maiti, 1997).
35S enhancer	Cauliflower mosaic virus 35S transcriptional enhancer region (Ow, 1987).
SMP promoter	Synthetic minimal plant promoter including the TATA box, an adenine-rich sequence involved in transcription initiation, from the Cestrum yellow leaf curling virus promoter (Stavolone, 2003b) linked to a sequence taken from the region that is 3′ to the TATA box of the 35S promoter (Ow, 1987).
TMV enhancer	The 5′ non-coding leader sequence (called omega) from tobacco mosaic virus (Gallie, 1987), which functions as a translational enhancer in plants (Gallie, 2002).
avhppd-03	The gene *avhppd-03*, derived from oat and codon optimized for enhanced expression, which encodes the enzyme AvHPPD-03. This enzyme catalyzes the formation of homogentisic acid, the aromatic precursor of plastoquinone and vitamin E biosynthesis (Matringe, 2005). In comparison with the native soybean HPPD, AvHPPD-03 has lower binding affinity for mesotrione, an herbicide that inhibits HPPD. Expression of *avhppd-03* in plant cells confers a tolerance to HPPD-inhibitor herbicides such as mesotrione.
NOS terminator	Terminator sequence from the nopaline synthase gene of A. tumefaciens (Accession No. V00087.1 (NCBI-Protein, 2012)). Provides a polyadenylation site (Depicker, 1982).

Genetic element	Description
pat-03-01 cassette	
35S promoter	Promoter region of cauliflower mosaic virus (Ow, 1987).
pat-03-01	Streptomyces viridochromogenes strain Tü494 gene, which encodes the selectable marker PAT. The native coding sequence (Wohllehen, 1988) was codon-optimized for enhanced expression. The synthetic gene *pat-03-01* was obtained from AgrEvo, Germany. PAT confers resistance to herbicides containing glufosinate-ammonium (phosphinothricin).
NOS terminator	Terminator sequence from the NOS gene of *A. tumefaciens* (Accession No. V00087.1 (NCBI-Protein, 2012)), provides a polyadenylation site (Depicker, 1982).
pat-03-02 cassette	
CMP promoter	Promoter and leader sequence from the Cestrum yellow leaf curling virus, similar to Accession No. AF364175.3 (NCBI-Protein, 2012) (Stavolone, 2003a).
TMV enhancer	The 5′ noncoding leader sequence (called omega) from tobacco mosaic virus (Gallie, 1987), which functions as a translational enhancer in plants (Gallie, 2002).
pat-03-02	*Streptomyces viridochromogenes* strain Tü494 gene, which encodes the selectable marker PAT. The native coding sequence (Wohllehen, 1988) was codon-optimized for enhanced expression and altered to remove restriction sites. PAT confers resistance to herbicides containing glufosinate-ammonium (phosphinothricin).
NOS terminator	Terminator sequence from the nopaline synthase gene of *A. tumefaciens* (Accession No. V00087.1 (NCBI-Protein, 2012), provides a polyadenylation site (Depicker, 1982).

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

The AvHPPD-03 protein expressed in SYHT0H2 soybeans is nearly identical to the microbially-produced from recombinant *Escherichia coli*, except for a minor four amino acid truncation in the *N*-terminus of the AvHPPD-03 protein expressed *in planta* (Syngenta and Bayer, 2012, p. 66). The PAT proteins, expressed by the genes *pat-03-01* and *pat-03-02*, derived from *Streptomyces viridochromogenes*, each encoding identical PAT amino acid sequences, are also identical in amino acid sequence to the PAT protein from SYHT0H2 soybeans.

The functional activities of the HPPD and PAT proteins were confirmed *in vivo* from field-grown SYHT0H2 soybeans (tables VI-1 [p. 66] and VII-1 [p. 81]). The applicants collected samples from leaves, roots, forage and seeds from five different growth stages (V4, V8, V10, R6, and R8), to quantify AvHPPD-03 and PAT proteins expression in SYHT0H2 soybeans. Both proteins were expressed at varying levels during all stages of the plant life cycle.

Detailed compositional and nutritional comparisons of SYHT0H2 soybeans and the conventional non-transformed soybean control Jack (Pp. 92-106), were conducted on samples collected from eight sites across the U.S. in 2010 (table X-5, p. 118). The analysis included moisture, protein, fat, ash, carbohydrates, amino acids, fatty acids, fibers, anti-nutrients, vitamin E isoforms, minerals and vitamins, consistent with OECD guidelines (OECD, 2001) (Syngenta and Bayer, 2012, table VIII-3, p. 94). SYHT0H2 soybeans had significantly lower (2.7%) potassium (K) and iron (Fe) content (3.5%) than the comparator Jack variety, but not significantly different from the reference varieties used in this study or from the International Life Sciences Institute' (ILSI) crop composition database (Syngenta and Bayer, 2012, table VIII-6, p. 99). The other compositional and nutritional components described above were not significantly different between SYHT0H2 soybeans and the conventional non-transformed soybean control Jack.

Tocopherols, substances produced by plants, play an important role as antioxidants. The amount and composition of tocopherols are regulated in part by biotic and abiotic factors surrounding the plants, such as developmental stage, stresses and nutrient availability (Tsegaye, 2002). α-tocopherol, an isoform with the highest amount of vitamin E activity, is an essential dietary component for mammals (Ujiie, 2005). The level of α-tocopherol in SYHT0H2 was significantly lower (11.6%) than in the non-transgenic parent line Jack, but the percentages of vitamin E in these two soybean varieties were within the concentration of tocopherols in the reference varieties. No differences were detected in the concentration of the other tocopherols (γ and δ) between Event SYHT0H2 and Jack soybean varieties (Syngenta and Bayer, 2012, table VIII-8, p. 100). Significantly higher amounts (1.3 – 3.6%) of the amino acids aspartic acid, threonine, serine, glutamic acid, proline, alanine, leucine, tyrosine, phenylalanine, lysine, histidine, and arginine, were detected in Event SYHT0H2 than in the comparator Jack variety. No significant differences were found in the concentration of the glycine, valine, methionine, isoleucine, and tryptophan amino acids between these two varieties (Syngenta and Bayer, 2012, table

VIII-9, p. 102). All the amino acid concentrations were found to be within the ranges of the reference varieties. As is the case of tocopherols, the levels of amino acids can be affected by abiotic factors (Wolf, 1982; Krishnan, 2005). Significantly higher amounts of palmitic, stearic, oleic, arachidic and behenic fatty acids, and significantly lower amounts of linoleic and linolenic fatty acids were obtained from SYHT0H2 soybean than from the comparator Jack variety. The concentrations of the fatty acids in Event SYHT0H2 and the conventional non-transformed soybean control Jack were within the ranges of the reference varieties and the ILSI crop composition database (Syngenta and Bayer, 2012, table VIII-10, p. 103). Fatty acid composition in soybeans can be significantly affected by abiotic factors (Tsukamoto, 1995).

Based on these results, it can be concluded that products derived from SYHT0H2 can be considered compositionally and nutritionally equivalent to those derived from convention soy.

D. Potential Impacts on Disease and Pest Susceptibilities

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 SYHT0H2 soybeans 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 or whether SYHT0H2 soybeans is likely to have significantly increased disease and pest susceptibility based on data and observations from field trials on specific pest and disease damage or incidence and any agronomic data that might relate to such damage. Impacts or changes are assessed to determine if they would (1) affect SYHT0H2 soybeans and/or result in significant introduction or spread of a damaging pest or disease to other plants; (2) result in the introduction, spread, and/or creation of a new disease; and/or (3) result in a significant exacerbation of a pest or disease for which APHIS has a control program. Any increase in pest or disease susceptibility is evaluated with respect to the context of currently cultivated varieties, the ability to manage the pest or disease, and the potential impact on agriculture.

SYHT0H2 soybeans were field tested in eight locations in the U.S. in 2010. Minimal damage was noted in SYHT0H2 soybeans and its control comparator Jack by the diseases frogeye leaf spot (*Cercospora sojina*) in IL, IA and IN trials, brown spot (*Septoria* sp.) in IA, powdery mildew (*Microsphaera diffusa*) in PA, and cercospora leaf spot (*Cercospora kukuchii*) in IA. The incidence of insects was also minimal during these trials finding light damage by green clover worms (*Plathypena scabra*) in IL and IA, bean leaf beetles (*Cerotoma trifurcata*) in IL, IA and IN, salt marsh caterpillars (*Estigmene acrea*) in MO, soybean looper (*Trichoplusia ni*) in IL, Japanese beetles (*Popillia japonica*) in IA, grasshoppers in IL, MO and IN, whiteflies in IL and PA, and leafhoppers in PA (p. 120).

Syngenta and Bayer aforementioned data indicate that SYHT0H2 soybeans are not biologically different from conventional soybeans (with the exception of the AvHPPD-03 and PAT proteins), and the herbicide resistant phenotypes did not alter the pest and disease incidences on SYHT0H2 soybeans; therefore, SYHT0H2 soybeans are no more susceptible to pests and diseases compared to conventional soybean cultivars.

E. Potential Impacts on Nontarget Organisms Beneficial to Agriculture

Event SYHT0H2 soybean is not engineered for insect pest resistance, thus there are no ‘target’ species, and thus no non-target species either. APHIS assessed whether exposure or consumption of Event SYHT0H2 soybeans would have an adverse effect on beneficial species or wildlife associated with soybeans. As discussed earlier, Event SYHT0H2 soybean is similar in nutritional and compositional analysis to unmodified control reference soybean varieties except for the intended changes in herbicide resistance associated with the production of AvHPPD-03 and PAT proteins in the plant.

The potential allergenicity and toxicity of introduced traits AvHPPD-03 and PAT proteins (obtained from *Avena sativa* and *Streptomyces viridochromogenes*, respectively) was assessed, according to the recommendations of the Codex Alimentarius Commission (Codex, 2003). The donor organism of the *avhppd-03* gene (oats) is not toxic and consumed by humans and animals alike (Mitchell, 2001), and the *pat* gene has a permanent exemption from food tolerances for PAT in all crops in the United States (EPA, 2002).

The bioinformatic analyses performed on the similarity of amino acid sequence of AvHPPD-03 demonstrated that it does not share structurally or immunologically relevant amino acid sequence similarities with known or putatively known toxins (Pp. 70-72, table VI-3), or known or putatively known allergens (Pp. 73-74). Additionally, digestive fate experiments with the AvHPPD-03 protein found that it was rapidly digested in simulated gastric fluid (SGF) by pepsin, a characteristic shared among many proteins with a history of safe consumption. The transiently stable protein fragments in the SGF assay were quickly degraded during a short exposure to simulated intestinal fluid (SIF). Rapid digestion of the full-length protein in SGF and SIF, together with rapid degradation of the transiently stable fragments from the SGF assay by SIF, indicates that it is highly unlikely that the AvHPPD-03 protein and its fragments will reach absorptive cells of the intestinal mucosa (p. 75). Finally, the AvHPPD-3 protein is present at very low concentrations in different SYHT0H2 tissues, which range between 5.8 to 59 µg/g of fresh tissue weight, in roots and V4 leaves, respectively (table VI-1, p. 66).

Syngenta and Bayer have submitted SYHT0H2 soybeans for regulatory approval by US Food and Drug Administration (FDA) as part of a voluntary consultation process, and appropriate regulatory agencies in another fourteen countries and regions. Any effects on non-target organisms that could potentially result from proposed changes in herbicide labels will be evaluated by the EPA. Based on the food and feed safety data, lack of toxicity and allergenicity of introduced gene products, APHIS concludes that feeding of Event SYHT0H2 soybean plant or seed by mammals and other nontarget organisms is unlikely to cause any adverse impact on their survival and reproduction.

F. Potential for Enhanced Weediness of SYHT0H2 Soybean

APHIS assessed whether SYHT0H2 soybeans is likely to become more weedy (i.e. more prevalent, competitive, damaging or difficult-to-control in situations where it is not wanted) than the nontransgenic progenitor from which it was derived, or other varieties of the crop currently under cultivation. The assessment considers the basic biology of the crop, the situations in which crop volunteers or feral populations are considered weeds, and an evaluation of the SYHT0H2 soybeans compared to the nontrangsenic progenitor or other appropriate counterpart evaluated under field (and/or lab) conditions characteristic for the regions of the United States where the SYHT0H2 soybeans is intended to be grown for characteristics related to establishment, competiveness, reproduction, survival, persistence and/or spread that could influence weediness and the ability to manage the crop as a weed.

Soybean is a highly domesticated legume species, and cultivated varieties of soybean in the US do not exhibit weedy characteristics, nor is soybean listed as a weed in any major weed references (Muenscher, 1952; Crockett, 1977; Holm et al., 1979). Likewise, soybean is not identified as a noxious weed in the Federal Noxious Weed List (7 CFR part 360; http://plants.usda.gov/java/noxious?rptType=Federal). Moreover, soybean does not possess any of the attributes commonly associated with weeds, such as long persistence of seed in the soil, the ability to disperse, invade, and become a dominant species in new or diverse landscapes, or the ability to compete well with native vegetation (Baker, 1965).

Phenotypic and agronomic characteristics of Event SYHT0H2 soybean were evaluated in a comparative manner to assess plant pest potential (OECD, 1993). These assessments included 16 plant growth and development characteristics: germination, dormancy and emergence, vegetative growth, reproductive growth, plant survival, seed dispersal, and ecological interactions (table X-1, Pp. 112-113). The following results were presented showing that Event SYHT0H2 soybean is phenotypically and agronomically similar to conventional Jack control and reference varieties:

- Seed dormancy is one of the potential traits effecting volunteerism and weediness. No significant differences were detected between Event SYHT0H2 soybean and the conventional control Jack (table X-3, p. 115). In all cases 96% of the seeds germinated under six different temperatures regimes in the tests. Although soybean seeds can potentially grow as volunteer plants in a subsequent crop rotation, volunteer plants would most likely be killed by frost in the soybean growing regions during autumn or winter of the year they were produced (OECD, 2000). Even if soybean volunteers get established, there are effective weed management strategies to control such volunteers (OECD, 2000; York, 2005).

- Another measure to demonstrate that a potentially enhanced reproductive advantage of Event SYHT0H2 over the conventional control Jack has not occurred, was an assessment of pollen size and viability (Pp. 115-116). Neither the diameter of pollen nor its viability were significantly different between Event SYHT0H2 over the conventional control Jack (table X-4, p. 116).

- The results of the 12 plant phenotypic characteristics measured in trials conducted in eight different locations in the US showed that the introduced trait did not unexpectedly alter the phenotypic or agronomic characteristics of Event SYHT0H2 soybean compared to conventional control Jack soybean. Early plant count, plant density, height, test weight, seed moisture, yield, seedling vigor, days to 50% flowering, flower color, lodging rating, pod shattering or days to maturity were not significantly different between Event SYHT0H2 over the conventional control Jack (tables X-6 and X-7, p. 119). These are indicative of no increased weediness of Event SYHT0H2 compared to the conventional control Jack soybean.

Results of these evaluations indicate that there is no fundamental difference between Event SYHT0H2 and the conventional control Jack for traits associated with weediness. Collectively, these findings support the conclusion that Event SYHT0H2 soybean is no more likely to be a weed compared to conventional control Jack soybean.

G. Potential Impacts on the Weediness of Any Other Plants with which It 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, 1991; Rieseberg, 1993; Soltis, 1993; Hegde, 2006), and even in the existing floras, the occurrence of hybridization or introgression is reported to be widespread (Stace, 1987; Rieseberg, 1993; Peterson, 2002). It has been a common practice by plant breeders to artificially introgress traits from wild relatives into crop plants to develop new cultivars. However, gene flow from crops to wild relatives is also thought of as having a potential to enhance the weediness of wild relatives, as observed in rice, sorghum, sunflower and few other crops (see Table 1 in Ellstrand, 1999).

Soybean is predominantly a self-pollinated species (OECD, 2000), yet a small amount of outcrossing does occur. Soybean typically exhibits a level of cross-pollination below one percent. Adjacent rows are measured to have between 0.03 and 3.62% outcrossing, but plants more than 4.5 meters apart cross at less than 0.02% (Caviness, 1966; Yoshimura, 2006). While most sources agree that insects do not greatly increase outcrossing rate in domestic soybean (Erickson, 1984), there is some evidence that some insects (notably honeybees) can increase hybridization rates (Free, 1970; McGregor, 1976). Pollen is only viable for 2-4 hours (it desiccates quickly) with anthesis (pollen shed) normally occurring in the late morning (Caviness, 1966). Current cultivation practices to prevent out-crossing have been deemed sufficient to prevent unwanted gene flow. For soybean, the Association of Official Seed Certifying Agencies (AOSCA) mandates a zero isolation distance where “Fields of soybeans shall be separated from any other variety or uncertified seed of the same variety by a distance adequate to prevent mechanical mixture”. Based upon these factors, it is unlikely that Event SYHT0H2 soybean will naturally outcross or hybridize to a significant extent with other soybean varieties in agricultural settings.

In assessing the risk of gene introgression from Event SYHT0H2 soybean into its sexually compatible relatives, APHIS considers two primary issues: 1) the potential for gene flow and introgression and, 2) the potential impact of introgression.

The genus *Glycine* is divided into two subgenera, *Glycine* and S*oja*. The subgenus *Soja* consists of three annual species: *G. soja* Sieb. and Zucc., the wild form of soybean; *G. gracilis* Skvortz., the weedy form of soybean; and *G. max*, the cultivated soybean. These species grow wild or semi-wild in Asia. Fertile hybrids between *G. max and G. soja* (Nakayama, 2002; Mizuguti, 2010), and between *G. max* and *G. gracilis* (Karasawa, 1952) occur. *Glycine soja* and *G. gracilis* grow naturally only in Asia, not in the United States (Lu, 2005). The subgenus *Glycine* consists of twelve wild perennial species. These species grow wild in Australia, South Pacific Islands and Asia (Newell, 1978), and do not exist naturally in the United States. Hybrids between perennial *Glycine* species are fertile.

Glycine max is the only *Glycine* species located in the United States, thus there are no other plant species with which *G. max* can interbreed (OECD, 2000). *Glycine max* has never been found in the wild (Hymowitz, 1987) without human intervention. Therefore, it is highly unlikely that soybean plants in the United States will be found outside of an agricultural setting. It is also highly unlikely that gene flow and introgression will occur between Event SYHT0H2 soybean and soybean plants in a natural environment. USDA has therefore determined that any adverse consequences of gene flow from Event SYHT0H2 soybean to wild or weedy species in the United States are highly unlikely.

H. Potential Changes to Agriculture or Cultivation Practices

APHIS considered whether there are likely to be significant changes to agricultural practices associated with cultivation of SYHT0H2 soybeans, and if so, are they likely to significantly exacerbate plant diseases or pests, especially those for which APHIS has a control program. Relative to currently cultivated soybean varieties, the only agricultural or cultivation practices that are expected to change if Event SYHT0H2 is no longer subject to regulation, are those related to weed management: in particular, the use of herbicides.

Some varieties of soybeans are tolerant to some of the approximately 23 herbicides registered for use in soybeans (Syngenta and Bayer, 2012, table XII-2, p. 131). Tolerance to certain kinds of herbicides, such glyphosate, has had an impact on higher adoption of conservation tillage in soybeans (Dill, 2008). Conservation tillage in soybeans can reduce the number of tillage operations, costs, and facilitate and improve weed control (USDA-NRCS, 2006). This tillage reduction decreases soil temperature that may be favorable for a higher incidence of certain pathogens. However, conservation tillage in soybeans does not significantly increase disease pressure in this crop (Sumner, 1981). Conservation tillage affects soil structure creating a habitat for an increased arthropod diversity. A higher number of arthropods in soybeans is not directly related with an increased arthropod pest pressure (Hammond, 1987; Stinner, 1990), therefore, planting SYHT0H2 soybeans is not expected to necessitate different cultivation practices, nor induce higher disease or pest pressure in this variety, compared with conventional soybean varieties. As described above, field studies with Event SYHT0H2 and conventional control Jack soybean demonstrated that neither the herbicide resistance traits nor the herbicide treatments appear to alter the response of these varieties to abiotic stress, diseases, or arthropod pests under natural levels of these stressors, nor were pest arthropods more abundant around Event SYHT0H2 plots.

In conclusion, as discussed throughout this document, SYHT0H2 soybeans is similar to conventional soybeans in its agronomic, phenotypic, environmental, and compositional characteristics and has levels of tolerance to insects and diseases comparable to conventional soybean varieties. Therefore, no significant adverse impacts on current agricultural or cultivation practices are expected following the introduction of Event SYHT0H2.

I. Potential Impacts from Transfer of Genetic Information to Organism with which SYHT0H2 Cannot Interbreed

The horizontal gene transfer (HGT) between unrelated organisms is one of the most intensively studied fields since 1940, and the issue gained extra attention with the release of transgenic plants into the environment (Dröge et al., 1998). HGT has been implicated as a major contributor to the spread of antibiotic resistance amongst pathogenic bacteria and the emergence of increased virulence in bacteria, eukaryotes, and viruses; and has contributed to major transitions in evolution. Gene exchange has been documented for nearly all types of genes and between unrelated organisms (Gogarten, 2002). For example, (Yoshida, 2010) through a comparative genomics analysis, implicated HGT for the presence of a similar genetic sequence between the parasitic plant purple witchweed (*Striga hermonthica*), which infests cereal fields (monocots), and sorghum (*Sorghum bicolor*).

APHIS examined the potential for the new genetic material inserted into Event SYHT0H2 soybean to be horizontally transferred to other organisms without sexual reproduction and whether such an event could lead directly or indirectly to disease, damage, injury or harm to plants. The Event SYHT0H2 soybean contains one coding sequence from oats, the modified *avhppd-03* gene and two from *Streptomyces viridochromogenes*, two *pat* genes, and two non-coding regulatory sequences from the bacteria, *Agrobacterium tumefaciens*. Horizontal gene transfer and expression of DNA from a plant species to other bacterial species is unlikely to occur based on the following observations.

Although there are many opportunities for plants to directly interact with fungi, bacteria, and parasitic plants (e.g. as commensals, symbionts, parasites, pathogens, decomposers, or in the guts of herbivores), so far there are no reports of significant horizontal gene transfer between sexually incompatible or evolutionarily distant organisms (as reviewed in Keese, 2008). Accumulated evidence show that there are universal gene-transfer barriers, regardless of whether transfer occurs among closely or distantly related organisms (Kaneko et al., 2000; Koonin et al., 2001; Wood et al., 2001; Kaneko, 2002; Brown, 2003; Sorek, 2007). Many genomes (or parts thereof) from bacteria that are closely associated with plants have been sequenced including *Agrobacterium* and *Rhizobium* (Kaneko et al., 2000; Wood et al., 2001; Kaneko, 2002). There is no evidence that these organisms contain genes derived from plants. 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). This is similar to the case in a recent report about of HGT between sorghum and purple witchweed. According to the authors (Yoshida, 2010), the incorporation of a specific genetic sequence occurred between sorghum and purple witchweed before speciation of purple witchweed (*S. hermonthica*) and related cowpea witchweed (*S. gesnerioides*), a parasitic plant of dicots, from their common ancestor. In other words, HGT is an extremely rare event, and a majority of those rare events occur over millions of years.

Transgene DNA promoters and coding sequences are optimized for plant expression, not prokaryotic bacterial expression. Thus even if horizontal gene transfer did occur, proteins corresponding to the transgenes are not likely to be produced. FDA has evaluated horizontal gene transfer from the use of antibiotic resistance marker genes, and concluded that the likelihood of transfer of antibiotic resistance genes from plant genomes to microorganisms in the gastrointestinal tract of humans or animals, or in the environment, is remote (FDA, 1998). Therefore APHIS concludes that horizontal gene transfer is unlikely to occur from Event SYHT0H2 soybean to microorganisms and thus no significant plant pest risk is expected from horizontal gene transfer.

J. Conclusion

APHIS has prepared this plant pest risk assessment in order to determine if Syngenta and Bayer Event SYHT0H2 soybean is unlikely to pose a plant pest risk. Due to the lack of plant pest risk from the inserted genetic material, the lack of weediness characteristics of Event SYHT0H2 soybean, the lack of atypical responses to disease or plant pests in the field, the lack of deleterious effects on non-targets or beneficial organisms in the agro-ecosystem, and the lack of horizontal gene transfer, APHIS concludes that Event SYHT0H2 soybean is unlikely to pose a plant pest risk.

APHIS has reviewed the information submitted in the petition, supporting documents, and other relevant information to assess the plant pest risk of SYHT0H2 soybean compared to the unmodified variety from which it was derived. APHIS concludes that SYHT0H2 soybean is unlikely to pose a plant pest risk based on the following findings:

- No plant pest risk was identified from the transformation process or the insertion of new genetic material because the inserted genetic material which was derived from plant pests does not result in the production of infectious agents or disease symptoms in plants.

- No increase in plant pest risk was identified in SYHT0H2 soybean from the expression of the inserted genetic material of new proteins, because SYHT0H2 soybean can be considered compositionally and nutritionally equivalent to those derived from convention soybeans.
- Disease and pest incidence and/or damage were not observed to be significantly increased or atypical in SYHT0H2 soybean compared to the nontransgenic counterpart or other comparators in field trials conducted in growing regions representative of where SYHT0H2 soybean is expected to be grown and greenhouse and laboratory studies. Observed agronomic traits also did not reveal any significant differences that would indirectly indicate that the SYHT0H2 soybean is more susceptible to pests or diseases.
- Exposure to and/or consumption of SYHT0H2 soybean are unlikely to have any adverse impacts on organisms beneficial to agriculture based on the analysis of the potential toxicity based on lack of amino acid sequence similarities with known toxins and mice acute toxicity studies and the donor organisms are widely distributed in nature.
- SYHT0H2 soybean is no more likely to become a weed or be weedier than conventional varieties of the crop based on its observed agronomic characteristics, weediness potential of the crop and current management practices available to control SYHT0H2 soybean as a weed.
- SYHT0H2 soybean is not expected to increase the weed risk potential of other species with which it can interbreed in the United States or its territories.
- Significant changes to agricultural or cultivation practices (e.g. pesticide applications, tillage, irrigation, harvesting, etc.) from adoption of SYHT0H2 soybean were not identified and not likely to increase plant diseases or pests or compromise their management.
- Horizontal gene transfer of the new genetic material inserted into SYHT0H2 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.

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