United States Department of Agriculture

Marketing and Regulatory Programs

Animal and Plant Health Inspection Service

Draft Plant Pest Risk Assessment for Double Herbicide-Tolerant Soybean (*Glycine max*) event FG72

Petition 09-328-01p

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Plant Pest Risk Assessment of Bayer CropScience Event FG72 Soybean

A. Introduction

Bayer CropScience (BCS) has petitioned APHIS for a determination that event FG72 double herbicide-tolerant soybean is unlikely to pose a plant pest risk (BCS, 2009) and, therefore, should no longer be a regulated article under APHIS' 7 Code of Federal Regulations (CFR) part 340. APHIS administers 7 CFR part 340 under the authority of the plant pest provisions of the Plant Protection Act of 2001[1]. This plant pest risk assessment was conducted to determine if FG72 soybean is unlikely to pose a plant pest risk. The event FG72 soybean (*Glycine max* cultivar Jack) expresses two added proteins which impart herbicide tolerance, 2mEPSPS from corn (*Zea mays*) and 4-hydroxyphenylpyruvate dioxygenase (HPPD), from *Pseudomonas fluorescens* (strain A32). The 2mEPSPS and HPPD proteins expressed in the soybean protect the plant from the application of the herbicides glyphosate and isoxaflutole (IFT), respectively.

FG72 soybean was produced by transformation of soybean tissue using direct gene transfer (biolistics). Because two of the regulatory sequences used to facilitate expression of the herbicide tolerance genes in FG72 soybean (Tobacco Etch Virus promoter and *A. tumefaciens nos* terminator) were derived from plant pests, the FG72 soybean has been considered a regulated article under APHIS regulations at 7 CFR part 340.

Potential impacts to be addressed in this risk assessment are those that pertain to the use of FG72 soybean and its progeny in the absence of confinement. APHIS uses data and information submitted by the applicant, in addition to current literature, to determine if FG72 soybean is unlikely to pose a plant pest risk. If APHIS determines that a GE organism is unlikely to pose a plant pest risk, then APHIS has no regulatory authority over that organism.

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

An analysis of agricultural or cultivation practices associated with FG72 soybean and impacts on the environment are considered in the Environmental Assessment (EA) for FG72 soybean. A thorough assessment of the effects of the determination on nontarget organisms, beneficial organisms and threatened and endangered species will also be considered in the EA.

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[1] Section 403 (14) of the Plant Protection Act (7USC Sec 7702(14)) defines plant pest as: "Plant Pest - The term "plant pest" means any living stage of any of the following that can directly or indirectly injure, cause damage to, or cause disease in any plant or plant product: (A) A protozoan. (B) A nonhuman animal. (C) A parasitic plant. (D) A bacterium. (E) A fungus. (F) A virus or viroid. (G) An infectious agent or other pathogen. (H) Any article similar to or allied with any of the articles specified in the preceding subparagraphs."

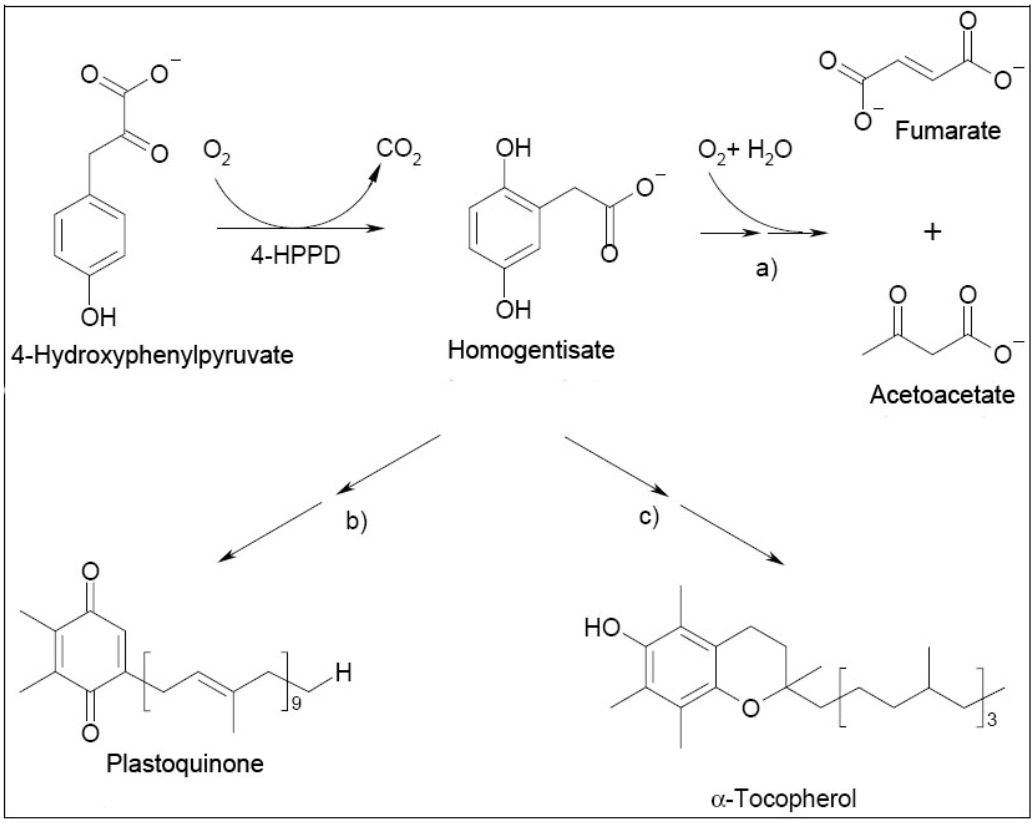
B. History of Development of Event FG72 Soybean

The first glyphosate tolerant soybean that was given a determination of nonregulated status in May, 1994 by APHIS was Roundup Ready® soybean 40-3-2 (OECD Unique Identifier MON-04032-6; submitted by Monsanto in petition number 93-258-01p). This event involved soybean that contained the DNA as follows: an enhanced 35S promoter from cauliflower mosaic virus, a chloroplast transit peptide from *Petunia hybrida* fused to the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) gene from *Agrobacterium* spp. strain CP4, and the nopaline synthase 3' terminator from *A. tumefaciens.* Monsanto completed its food safety consultation with the U.S. Food and Drug Administration (FDA) on soybean Event 40-3-2 on January 27, 1995 (http://www.accessdata.fda.gov; BNF 00001). In September 1993, the Environmental Protection Agency (EPA) completed its Reregistration Eligibility Decision (RED) on the pesticide active ingredient glyphosate (EPA-738-F-93-011). In 1995, the Plant Biosafety Office (PBO) of the Canadian Food Inspection Agency (CFIA) concluded that Monsanto's Event 40-3-1 soybean was substantially equivalent to currently grown soybean, in terms of their potential environmental impact and livestock feed safety and the novel traits would not have any substantial negative effect on the environment (CFIA Decision Document DD95-05; http://www.inspection.gc.ca). In 2006, Monsanto developed a second generation glyphosate-tolerant soybean called RReady2Yield™, Event MON 89788. APHIS made a determination of nonregulated status for Event 89788 (RReady2Yield™) on July 23, 2007. EPA and FDA did not re-evaluate MON 89788 because the gene of interest was identical to Event 40-3-2. MON 89788 is very similar to MON-04032-6. Both plants were genetically engineered to be glyphosate tolerant by inserting a gene (from *Agrobacterium* spp. strain CP4) coding for *epsps* into the soybean genome. The three major differences between MON 89788 and MON-04032-6 are: the use of a different promoter for the *cp4 epsps* gene, a different transformation method, and the use of a different recipient variety. The Food Standards Australia New Zealand (FSANZ) approved MON 89788 soybean in a pre-market safety assessment on August 8, 2007 (Application A592) for import (but not for cultivation).

There are no historical genetically engineered events for the insertion of the *hppd* gene into soybean or any other crop species. Page 33 of the BCS submitted petition describes the action of the protein made by the *hppd* gene along with diagram for the two metabolic pathways in plants; the biosynthesis of plastoquinone and biosynthesis of tocopherol:

> "The biochemical pathways in which HPPD is involved differ between plants and nonphotosynthetic organisms. In bacteria and animals, it merely serves catabolic purposes by catalyzing the first committed step in tyrosine degradation that in the end yields energetically exploitable glucogenic and ketogenic products (Brownlee, Johnson-Winters et al., 2004). In plants, however, it is also involved in several anabolic pathways; its reaction product homogentisate (2,5-dihydroxyohenylacetate) being the aromatic precursor of tocopherol and plastoquinone, which are essential to the photosynthetic transport chain and antioxidative systems (Fritze, 2004)."

Figure 1. Biochemical pathways of HPPD proteins



a) catabolism of tyrosine; b) biosynthesis of plastoquinone (plants); c) biosynthesis of tocopherol (plants)

The first herbicide developed in this family was isoxaflutole by Rhone Poulenc (now Aventis) and was registered for use in corn in 1998 by the EPA (FR Vol. 63, No.104 50773).

From the EPA Isoxaflutole Fact Sheet (EPA, 1998):

> "Isoxaflutole is a pigment inhibitor. It works by preventing the biosynthesis of carotenoid pigments, which protect chlorophyll from decomposition by sunlight. Without carotenoid pigments, chlorophyll pigments are photo-oxidized and chloroplasts break down. Without the energy collecting action of the chlorophyll, the whole plant eventually dies."

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 (Figure 1). Plastoquinone is a carotenoid pigment that is needed for electron transport process of photosynthesis (http://www.uniprot.org/keywords/618).

HPPD controls a broad spectrum of grass and broadleaf weeds and is effective on difficult to control weeds such as velvetleaf and wooly cupgrass (Zollinger, 2009). HPPD is called a proherbicide because it degrades rapidly to the active molecule diketonitrile. The primary

breakdown product is mobile and breaks down more slowly than isoxaflutole itself (Zollinger, 2009). DKN is reactivated by rainfall events, providing control of small weeds that have emerged. 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, Oliveira et al., 2009). Because of the potential for leaching, especially on permeable soils, isoxaflutole is a restricted-use pesticide. The label prohibits 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 because this is the site of new carotenoid synthesis (Zollinger, 2009).

Since the FG72 soybean is tolerant to isoxaflutole, this herbicide can be applied prior to planting (pre-plant) without any significant damage to the plant while being toxic to weeds not managed by glyphosate.

Bayer CropScience has now developed the transgenic soybean, Event FG72, which expresses both 2mEPSPS protein and HPPD proteins. Event FG72 soybean has been developed by BCS as an alternative soybean product that could help manage herbicide resistant weed populations.

Event FG72 soybean has been field tested under APHIS regulations since 2001. Data were provided in the petition for field trials completed prior to the petition submission. Field test reports can be found in the BCS Event FG72 soybean petition (BCS, 2009, pp. 93).

BCS will make a request to the Environmental Protection Agency (EPA) to allow the use of an isoxaflutole formulation to be used on Event FG72 soybean. BCS initiated the consultation process with FDA for the commercial distribution of FG72 soybean and submitted a safety and nutritional assessment of food and feed derived from FG72 soybean to the FDA on December 3, 2009. FDA is currently evaluating the submission, and as of March 16, 2012, has not completed the consultation.

C. Description of the Modifications

Event FG72 soybean contains the stably integrated genes *2mepsps* and *hppdPfW336*, which encode respectively the 2mEPSPS and HPPD proteins. The *2mepsps* and the *hppdPfW336* genes were introduced into the soybean genome by means of direct gene transfer (biolistics). “Event FG72 contains a single insert of two consecutive complete copies of the linear fragment.” (BCS, 2009, pp.25).

BCS’ double herbicide-tolerant soybean (Event FG72) has been genetically engineered to contain the following transgene fragments:

The *2mepsps* gene expression cassette borne by pSF10 is represented by the following string: “Ph4a748-intron1 h3At-TPotpC::*2mepsps*::3’histonAt” (BCS, 2009, pp.22):

Ph4a748 promoter and intron 1 h3At: The Ph4a748 promoter sequence is derived from the histone H4 gene of *Arabidopsis thaliana* (Chabouté, Clement et al., 1987) and controls expression of the *2mepsps* gene. The Ph4a748 promoter, combined with the first intron of gene II of the histone H3.III variant of *Arabidopsis thaliana* (Chaubet, Clement et al., 1992) directs high level constitutive expression, especially in rapidly growing plant tissues.

TPotp C transit peptide: The optimized transit peptide, which contains sequences from the RuBisCO small subunit genes of corn and sunflower, targets the mature protein to the plastids, which is where the wildtype protein would be located (Lebrun, Leroux et al., 1996).

***2mepsps* coding sequence:** The wildtype *epsps* gene isolated from maize (*Zea mays*) was mutated using site-directed mutagenesis. Two point mutations resulted in the double mutant *2mepsps* gene (Lebrun, Sailland et al., 1997). A methionine codon is added to the N-terminal of the 2mEPSPS protein sequence in order to restore the cleavage site of the optimized plastid transit peptide. The *2mepsps* gene encodes a 47 kDa protein consisting of 445 amino acids.

3'histonAt terminator: The 3' untranslated region of the histone from *Arabidopsis thaliana* (Chabouté, Clement et al., 1987).

The *hppdPfW336* gene expression cassette is represented by the following string: "Ph4a748 ABBC-5'tev -TPotpY::*hppdPfW336*::3'nos" (p.23, BCS Event FG72 petition:

Ph4a748 ABBC promoter and 5'tev enhancer: The same Ph4a748 promoter was used to drive the expression of the *hppdPfW336* gene, but an internal portion of the promoter sequence (referred to as "B") was duplicated to increase the promoter activity in plant cells. In combination with the leader sequence of the tobacco etch virus (5' tev; Carrington and Freed, 1990), this promoter brings the level of expression of hppdPfW336 gene to an appropriate level that enables tolerance at agronomic doses of IFT.

TPotp Y transit peptide: The optimized transit peptide, which contains sequences from the RuBisCO small subunit genes of corn and sunflower, targets the mature protein to the plastids, which is where the wildtype protein would be located (Lebrun, Leroux et al., 1996).

***hppdPfW336* gene coding sequence:** The wildtype *hppd* gene isolated from *Pseudomonas fluorescens* was mutated using site directed mutagenesis. A point mutation resulted in the *hppdPfW336* gene (Boudec, Rodgers et al., 2001). The *hppdPfW336* gene encodes a 40 kDa protein consisting of 358 amino acids.

3' nos terminator: The 3' untranslated region of the nopaline synthase from *Agrobacterium tumefaciens* is a polyadenylation signal (Depicker, Stachel et al., 1982).

D. Plant Pest Risk Assessment

This plant pest risk assessment is to determine whether BCS Event FG72 is unlikely to pose a plant pest risk. If APHIS determines that a GE organism is unlikely to pose a plant pest risk, APHIS then has no regulatory authority over that organism under 7 CFR part 340.

APHIS administers the regulations 7 CFR part 340 under the authority of the Plant Protection Act of 2000 (PPA).

The Animal and Plant Health Inspection Service of the United States Department of Agriculture (USDA-APHIS) has prepared a Plant Pest Risk Assessment in response to a petition (APHIS Number 09-328-01p) from BCS. APHIS regulation 7 CFR 340.6(c) stipulates the information needed for consideration in a petition for nonregulated status. APHIS evaluated information submitted by the applicant related to plant pest risk characteristics, disease and pest susceptibilities, expression of the gene product, new enzymes, or changes to plant metabolism, potential impacts on non-target organisms, weediness of the regulated article, and any impacts on the weediness of any other plant with which it can interbreed. Issues related to agricultural or cultivation practices are considered in the Environmental Assessment for BCS Event FG72 soybean.

Based on information on the biology of soybean (OECD, 2000), data presented by BCS (BCS, 2009, Appendix I, pp. 93-114) and scientific data relevant to a discussion of plant pest risk, APHIS concluded the following regarding BCS double herbicide-tolerant soybean Event FG72:

Potential Impacts of Genetic Modifications on Disease and Pest Susceptibilities

USDA-APHIS assessed whether BCS Event FG72 soybean is likely to have significantly increased disease and pest susceptibility. The assessment encompasses a thorough consideration of introduced traits, their impact on agronomic traits and plant composition, and interactions with pests and diseases.

BCS observed field trials for damage due to diseases (Alternaria leaf spot, Anthracnose, Asian rust, Bacterial blight, Bacterial pustule, Brown spot, Brown stem rot, Cercospora leaf blight, Charcoal rot, Downy mildew, Frogeye leaf spot, *Fusarium*, *Phytophthora*, Powdery mildew, *Pythium*, *Rhizoctonia*, *Sclerotinia*, *Septoria*, Soybean mosaic virus, Soybean rust, Stem canker, Sudden death, White mold) and insect pests (aphid, bean leaf beetle, blister beetle, corn rootworm, flea beetle, grasshopper, green cloverworm, Japanese beetle, leafhopper, leafroller, Mexican bean beetle, spidermite, seed corn maggot, soybean stem borer, stink bug, tarnished plant bug, thistle caterpillar, thrips, velvetbean caterpillar, white fly, wireworm, and woolybear caterpillar) for four consecutive years conducted across different agroecological conditions (from 2001-2004) and then for three years (2007-2009). Data were collected on pest and disease damage across 15 sites from 2001-2003 and 18 sites from 2007-2009. The observations submitted by BCS indicated no meaningful differences between Event FG72 soybean and the non-transgenic counterparts for diseases or insect pests (BCS, 2009, Section VII.D, Table 22). Thus Event FG72 is expected to be susceptible to the same plant pathogens and insect pests as conventional soybean with the noted exceptions listed in the variety registration for Jack. From the BCS petition (pp. 65) “The variety registration of Jack, claims resistance to soybean cyst nematode (SCN) (Races 3 and 4) *Heterodera glycines* Ichinohe) and susceptibility to

Phytophthora rot (Races 1, 4, and 7) caused by *Phytophthora megasperma* (Drechs.) f. sp. *glycinea* T. Kuan & D.C. Erwin. Neither of these phytopathologies was observed consistently in the trials, so it was not possible to confirm xpression of these variety traits. At the Perry location, one plant of event FG72 was presumed to have died of *Phytophthora* root rot."

BCS' aforementioned data indicate that Event FG72 soybean is not biologically different from conventional soybean (with the exception of the HPPD and 2mEPSPS proteins) and the herbicide resistant phenotypes did not alter the pest and disease incidences on Event FG72 soybean; therefore, Event FG72 soybean is no more susceptible to pests and diseases compared to conventional soybean cultivars.

Expression of the Gene Product, New Enzymes, or Changes to Plant Metabolism

The HPPD and 2mEPSPS proteins expressed in Event FG72 soybean are nearly identical to the native proteins produced by *Pseudomonas fluorescens* and corn, respectively. The HPPD protein was modified by the replacement of the amino acid glycine with a tryptophan and the 2mEPSPS protein has two point mutations along with a methionine codon added to the N-terminal end.

The functional activities of the HPPD and 2mEPSPS proteins were confirmed *in vivo* from field-grown Event FG72 soybean plants (BCS, 2009, pp. 38). BCS collected samples from grain, seeds, leaf samples from three different growth stages (V4, V6, V8), stem and root at V4 and V8 stages to quantify HPPD and 2mEPSPS protein expression in Event FG72 soybean (BCS, 2009, Appendix 2). Both proteins were expressed at varying levels during all stages of the plant life cycle (BCS, 2009, Table 7 and 8).

USDA-APHIS assessed whether changes in plant metabolism or composition in Event FG72 soybean is likely to alter plant pest risk. The assessment encompasses a consideration of the expressed added proteins or enzymes and their effect on plant metabolism and an evaluation of whether the nutrients and anti-nutrient levels in harvested seed and forage derived from Event FG72 soybean are comparable to those in the non-transgenic parent line, Jack variety as well as three commercial soybean varieties, Stine 2686-6, 2788 and 3000-0. (BCS, 2009, Tables 25-29, pp. 71-74).

Detailed compositional and nutritional comparisons of Event FG72 soybean and the conventional soybean control, Jack, were conducted on samples collected from ten sites across the U.S. in 2008 and compared against reference ranges calculated from three commercial soybean varieties listed in the above paragraph. The analysis included moisture, protein, fat, ash, carbohydrates, amino acids, fatty acids, anti-nutrients and isoflavones, consistent with OECD guidelines (OECD, 2001).

Appendix 2.G (BCS, 2009, pp. 134) describes the materials and methods used for the compositional analysis. The overall data set was examined for evidence of biologically relevant changes. All Event FG72 soybean compositional analysis values were similar to the two test and control entries and fell within the commercial variety and literature reference ranges (BCS, 2009, Tables 25-29, pp. 71-74). The compositional analyses confirmed that Event FG72 soybean is compositionally equivalent to the three conventional soybean lines (Stine 2686-6, 2788 and

3000-0) with no single data entry outside any reference ranges (commercial varieties or literature references). Collectively, the compositional data support the conclusion that Event FG72 soybean does not have biologically meaningful differences from conventional soybean from a food/feed safety and/or nutritional perspective.

Based on all the above noted considerations, APHIS concludes that Event FG72 soybean poses no more of a plant pest risk from new gene products, changes to plant metabolism or composition (with the exception of intended changes in herbicide resistance) than conventional soybean.

Weediness of Event FG72 Soybean

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, Pancho et al., 1979). Likewise, soybean is not identified as a noxious weed in the Federal Noxious Weed List (7 CFR § 360; http://plants.usda.gov/java/noxious?rptType=Federal). Moreover, soybean does not possess any of the attributes commonly associated with weeds (Baker, 1965), 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.

Phenotypic and agronomic characteristics of Event FG72 soybean were evaluated in a comparative manner to assess plant pest potential (OECD, 1993). These assessments included 17 plant growth and development characteristics: early stand count, plant vigor, days to flowering, flower color, leaf shape, health rating at stage V4-5, health rating at stage R1, health rating of mature plants, pubescence color, pod color, hilum color, canopy, days to maturity, yield in bu/ac, lodging, final stand count and pod shatter (BCS, 2009, Table 57, pp. 181). BCS presented the following results to show that Event FG72 soybean is phenotypically and agronomically similar to conventional control reference varieties:

- Seed dormancy is one of the potential traits effecting volunteerism and weediness. No statistically or biologically significant differences were detected between Event FG72 soybean and the conventional control (BCS, 2009, Table 24, pp. 64). Seed tests were completed by Iowa State Seed Lab using standard testing (BCS, 2009, pp. 133). No dormant seeds were identified. There was a slight difference (94% vs. 96%) in germination at day 6 (BCS, 2009, Table 24, pp. 64) between Event FG72 and the parent line Jack, but when germination was extended to 13 days, the percent viable seed of the samples (95% vs. 96%) were statistically similar. The germination values of Event FG72 soybean were not different from the conventional control and were within the range of accepted germination values for certified soybean seed. 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. Even if soybean volunteers get established, there are effective weed management strategies to control such volunteers (OECD, 2000; York, Beam et al., 2005).

- The results of the 17 plant growth and development characteristics showed the introduced trait did not unexpectedly alter the phenotypic or agronomic characteristics of Event FG72 soybean compared to conventional soybean. The early plant density and plant vigor ratings were lower in unsprayed Event FG72 plots than in commercial variety plots which carried through to late season possibly due to seed lot quality. The days to 50% flowering were shorter for Event FG72 compared to commercial varieties, but for days to 95% flowering, there were no differences between the Event FG72 soybean and commercial varieties. There are indicative of no increased weediness of Event FG72 soybean (BCS, 2009, pp. 53-68).

Results of these evaluations indicate that there is no fundamental difference between Event FG72 soybean and the conventional control for traits associated with weediness. Collectively, these findings support the conclusion that Event FG72 soybean is no more likely to be a weed compared to conventional soybean.

Impacts on the Weediness of Any Other Plant with which Event FG72 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, 1991; Rieseberg and Wendel, 1993; Soltis and Soltis, 1993; Hegde, Nason et al., 2006), and even in the existing floras, the occurrence of hybridization or introgression is reported to be widespread (Stace, 1987; Rieseberg and Wendel, 1993; Peterson, Pearman 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. 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, Prentice et al., 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, Matsuo et al., 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 FG72 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 FG72 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 and Yamaguchi, 2002; Mizuguti, Ohigashi et al., 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 and Hymowitz, 1978), and do not exist naturally in the US. 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. *Glycine max* has never been found in the wild (Hymowitz and Singh, 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 FG72 soybean and soybean plants in a natural environment. USDA has therefore determined that any adverse consequences of gene flow from Event FG72 soybean to wild or weedy species in the United States are highly unlikely.

Effects of Event FG72 Soybean on Non-target Organisms

Event FG72 soybean is not engineered for insect pest resistance, thus there are no 'target' species, and thus no 'nontarget' species either. APHIS assessed whether exposure or consumption of Event FG72 soybeans would have an adverse effect on beneficial species or wildlife associated with soybeans. As discussed earlier, Event FG72 soybean is similar in nutritional and compositional analysis to unmodified control soybean variety except for the intended changes in herbicide resistance associated with the production of HPPD and 2mEPSPS proteins in the plant.

BCS also assessed the potential allergenicity and toxicity of introduced traits (HPPD and 2mEPSPS proteins obtained from *Pseudomonas fluorescens* and corn, respectively) according to the recommendations of the Codex Alimentarius Commission (Codex, 2003). The donor organism of the *2mepsps* gene (corn) is not toxic and consumed by humans and animals alike and the donor organism of the *hppd* gene, *Pseudomonas fluorescens*, can be an opportunistic pathogen in immunocompromised patients, but is generally seen as having poor virulence due to its inability to multiply at body temperature and having to compete against host defenses (Liu, 1964).

BCS's bioinformatic analyses of both proteins demonstrated that they do not share structurally or immunologically relevant amino acid sequence similarities with known allergens. Additionally, BCS conducted digestive fate experiments with the HPPD and 2mEPSPS proteins and found that the full-length proteins are rapidly digested in simulated gastric fluid (SGF), 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 proteins 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 HPPD and 2mEPSPS proteins and their fragments will reach absorptive cells of the intestinal mucosa. Finally, the HPPD and 2mEPSPS proteins are present at very low concentrations which average no more than 0.00023% and 0.041%, respectively, of the total protein in Event FG72 soybean mature seed.

BCS also assessed both proteins for their potential toxicity. HPPD is ubiquitous in nature across all kingdoms including bacteria, fungi, plants and animals. HPPD has been characterized in organisms present in human food such as carrot, barley, pork and beef (BCS, 2009, pp. 38). The proteins lack structural similarity to known toxins or biologically active proteins known to have adverse effects to mammals. Both proteins occur at extremely low levels in the harvested seed (see above paragraph for concentrations), in other words, they make up a negligible portion of the total protein present in food and feed derived from Event FG72 soybean. BCS provided data showing that feeding high rates of the HPPD and 2mEPSPS proteins purified from Event FG72 soybean immature seed did not cause adverse effects on mice (BCS, 2009, pp. 47-52).

Furthermore, BCS has submitted food and feed safety data to FDA as part of a voluntary consultation process. Based on the food and feed safety data, lack of toxicity and allergenicity of introduced gene products, APHIS concludes that feeding of Event FG72 soybean plant or seed by mammals and other nontarget organisms is unlikely to cause any adverse impact on their survival and reproduction.

Transfer of Genetic Information to Organisms with which Event FG72 Soybean 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, Puhler 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, Doolittle et al., 2002). For example, recently, Yoshida and colleagues (Yoshida, Maruyama et al., 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 FG72 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 FG72 soybean contains one coding sequence from bacteria, the modified *hppdPfW336* gene from *Pseudomonas fluorescens* (as described by Boudec, Rodgers et al., 2001) 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, Nakamura et al., 2000; Koonin, Makarova et al., 2001; Wood, Setubal et al., 2001; Kaneko, Nakamura et al., 2002; Brown, 2003; Sorek, Zhu et al., 2007). Many genomes (or parts thereof) from bacteria that are closely associated with plants have been sequenced including *Agrobacterium* and *Rhizobium* (Kaneko, Nakamura et al., 2000; Wood, Setubal et al., 2001; Kaneko, Nakamura et al., 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, Makarova 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, Maruyama et al., 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 FG72 soybean to microorganisms and thus no significant plant pest risk is expected from horizontal gene transfer.

E. Conclusion

APHIS has reviewed and conducted a plant pest risk assessment on BCS Event FG72 soybean. Due to the lack of plant pest risk from the inserted genetic material, the lack of weediness characteristics of BCS Event FG72 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 BCS Event FG72 soybean is unlikely to pose a plant pest risk.

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