Genective Petition (11-342-01p) for Determination of Non-regulated Status of Event VCO-Ø1981-5 Corn

OECD Unique Identifier: VCO-Ø1981-5

Plant Pest Risk Assessment

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A. Introduction

Genective SA in collaboration with Athenix Corp., now an affiliate of Bayer CropScience LP, has developed herbicide-tolerant Event VCO-Ø1981-5 corn. Genective SA (referred to as "Genective" hereafter) has petitioned APHIS (USDA-APHIS Petition Number #11-342-01p) for a determination that genetically engineered (GE) corn (*Zea mays*) Event VCO-Ø1981-5 is unlikely to pose a plant pest risk and, therefore, is no longer a regulated article under regulations at 7 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]. The Genective petition request for Event VCO-Ø1981-5 corn was originally received on December 8, 2011. A revised version of the petition, received March 28, 2012, was deemed technically complete and is the version of the petition referenced in this document. This plant pest risk assessment was conducted to determine whether Event VCO-Ø1981-5 corn is likely to pose a plant pest risk.

B. Development of Event VCO-Ø1981-5 Corn

Corn (*Zea mays*) is among the most important crops in the United States, with more than 96 million acres cultivated for all purposes in 2012(USDA-NASS 2012). The United States is the largest producer of corn in the world (USDA-ERS 2010). Corn has been used by U.S. farmers as the primary feed grain, accounting for more than 90 percent of the total value of feed grains (USDA-ERS 2010). Corn is also considered a major biofuel crop in the U.S. Recent strong demand for ethanol production has resulted in increased corn demand and higher corn prices and has provided incentives to increase corn acreage. USDA has projected that U.S. corn cultivation may continue to increase in the coming year (USDA-NASS 2012). Most recently in the U.S., farmers planted 88 percent of their acreage with biotechnology-derived varieties. Biotechnology-derived traits in these genetically engineered (GE) varieties consist of: 21% herbicide tolerance, 15% insect resistance and 52% stacked varieties containing both insect and herbicide resistance (USDA-NASS 2012).

Weed competition can be a major limiting factor in corn production leading to significant yield and quality reductions by direct competition for light, water and nutrients in the soil. Late-season weed infestations do not reduce corn yield nearly as much as early-season weed competition; however, late-season weeds can harbor destructive insect pests (UC-IPM 2009). In 2011, approximately 72% of corn planted in the U.S. possessed tolerance to an herbicide that was conferred through biotechnology (USDA-ERS 2011). The primary herbicide tolerance trait in use has been glyphosate tolerance, and the adoption of this trait in soybeans is even higher (94%). The wide-spread adoption of herbicide –tolerant crops, especially GE-derived glyphosate-tolerant crops, has changed the approach that farmers take to avoid yield losses from weeds (Duke and Powles, 2009; Gianessi, 2008). Most transgenic herbicide tolerant corn has been developed by the

[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."

insertion of genes that transfer tolerance for glyphosate, which expands the control options for annual and perennial grass and broadleaf weeds.

Glyphosate (*N*-(phosphonomethyl) glycine) is a broad-spectrum systemic herbicide used to kill weeds. It is registered with the Environmental Protection Agency (EPA) for non-selective weed control for both non-food use and food use plants. Glyphosate's mode of action is to inhibit the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) involved in the synthesis of the aromatic amino acids: tyrosine, tryptophan and phenylalanine. Glyphosate functions due to its resemblance to the structure of the substrate for EPSPS enzyme and thereby competing with this substrate for the enzyme's active site, thus preventing the synthesis of aromatic amino acids and killing the plant.

The first corn line containing a glyphosate tolerance trait produced through the use of biotechnology was granted nonregulated status in 1995 and since that time several other corn lines containing glyphosate tolerance have also been granted nonregulated status by APHIS-USDA. APHIS BRS completed Plant Pest Risk Assessments (PPRA) and Environmental Assessments (EAs) for glyphosate tolerant corn in a number of petitions (http://www.aphis.usda.gov/biotechnology/not_reg.html). The EAs fully addressed all environmental areas of potential concern. In these petitions, APHIS concluded on the basis of the EA that the impacts from making a determination of nonregulated status would not be significant. The agency issued Findings of No Significant Impact (FONSI) and made determinations of nonregulated status for each. The 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) proteins from various GE crops have completed consultations procedures by Office of Premarket Approval, Center for Food Safety and Applied Nutrition, Food and Drug Administration (FDA) since 1994 (US-FDA 2013). To date, regulatory authorities in twelve countries have approved the environmental (commercial) release of at least 30 GE plant lines that contain non-plant derived EPSPS proteins. These are represented in a total of seven plant species: *Beta vulgaris* L. (sugar beet), *Brassica napus* L. and *Brassica rapa* L. (oilseed rape and turnip rape, respectively, although both can be referred to as canola) *Glycine max* L. (soybean), *Gossypium hirsutum* L. (cotton), *Medicago sativa* L. (alfalfa) and *Zea mays* L. (maize) (ILSI 2010).

Genective has developed corn Event VCO-Ø1981-5 which is designed to provide growers with weed control through over-the-top glyphosate application. Event VCO-Ø1981-5 corn expresses a version of 5-enolpyruvylshikimate-3-phosphate synthase protein derived from the common soil bacterium *Arthrobacter globiformis* (Genective 2011, p. 34) that is resistant to glyphosate. Genective conducted confined field tests of Event VCO-Ø1981-5 corn in the United States and its territories since 2007 and in Europe and Canada since 2009. The agronomic evaluations have been conducted by Genective scientists and data in the petition are from those confined field trials.

Description of the Modification—Genetic material inserted and protein produced

Genective corn Event VCO-Ø1981-5 was produced using *Agrobacterium tumefaciens*-mediated transformation of the hybrid corn line Hi-II from Maize Genetics Cooperation Stock Center (Genective 2011, page 19). The *A. tumefaciens* strain LBA4404 that was used to develop Event VCO-Ø1981-5 was made non-pathogenic by removing tumor

inducing (Ti) DNA sequences normally present in *A. tumefaciens* (Koncz and Schell 1986). The disarmed *A. tumefaciens* carried a binary plasmid vector pAG3541containing the *epsps greg23ace5* gene (Genective 2011, page 19). *In vitro* selection of transformation events was based on tolerance to the herbicide glyphosate which is rendered non-toxic to plant tissues by the presence of the EPSPS protein. The size of the T-DNA was 3730 base pairs (bp) and contained a single T-DNA delineated by left and right border regions in which there was one expression cassette: *epsps grg23ace5*.

The *epsps grg23ace5* expression cassette consisted of the following genetic elements (Genective 2011, pp. 23-25):

- Border sequences: Left and right border sequences contain 25 base pairs of DNA from *A. tumefaciens* to facilitate T-DNA transfer (Depicker *et al.* 1982; Barker *et al.* 1983)
- ScUbi4 Promoter: Promoter region of the ubiquitin-4 gene from *Saccharum officinarum* L. (sugarcane) (Albert and Wei 2003) that directs transcription in plant cells.
- ScUbi4 5' UTR: 5'untranslated region of the ubiquitin-4 gene from *Saccharum officinarum* L. (sugarcane) (Albert and Wei 2003) that helps regulate gene expression
- ScUbi4 intron: Intron region of the ubiquitin-4 gene from *Saccharum officinarum* L. (sugarcane) (Albert and Wei 2003) mAHAS chloroplast transit peptide: N-terminal chloroplast transit peptide sequence derived from the *Zea mays* L. acetohydroxyacid synthase (*ahas*) gene (Fang *et al*. 1992) that directs transport of the expressed protein to the chloroplast.
- *epsps grg23ace5* coding sequence: Codon-optimized version of the *epsps grg23ace5* gene from *Arthrobacter globiformis* encoding EPSPS ACE5 protein (Schouten *et al*. 2010)
- 35S terminator: Terminator region of the 35S transcript of the Cauliflower Mosaic Virus, which terminates mRNA transcription and induces polyadenylation (Gardner *et al*. 1981)

In addition to the above-mentioned genetic elements, the inserted T-DNA also contains short noncoding intervening DNA sequences. The intervening sequences contain restriction enzyme recognition sites and are used for cloning purposes.

Data provided from Genective and reviewed by APHIS demonstrated:

- The T-DNA inserted into the corn genome is present at a single locus and contains a single copy of the inserted transgene (Genective 2011, Appendix 3);
- No region from the genetic elements outside of the T-DNA borders from the transformation vector was inserted (Genective 2011, Appendix3);
- A short deletion (21bp) in the Event VCO-Ø1981-5 genome was created by insertion of the transgene. Genective has identified 12 potential open reading frames (ORFs) created in Event VCO-Ø1981-5. From Bioinformatics analysis, no homologies with known allergen sequences were identified and no significant homologies with known toxins or other harmful proteins were found;

- Short deletions at the extremities of the T-DNA borders (22 bp on the right and 16 bp on the left borders) resulted in the insert size being 3692 bp;
- All inserted regulatory sequences and the organization of the inserted gene in Event VCO-Ø1981-5 are intact and identical to their original arrangements in the donor plasmid pAG3541;
- The stability of the introduced genes was determined by event-specific and gene-specific endpoint PCR analyses for four generations (Genective 2011, pp.26-29). The stability integration was further confirmed by the Mendelian inheritance of the T-DNA in Event VCO-Ø1981-5 corn over four generations (Genective 2011, p. 29)

C. Potential for Event VCO-Ø1981-5 Corn to have Altered Disease and Pest Susceptibilities

USDA-APHIS assessed whether Event VCO-Ø1981-5 corn is likely to have significantly increased disease and pest susceptibility. This assessment encompasses a thorough consideration of the transformation process, introduced genes and their genetic elements, and their expression products to cause interactions with pests and diseases.

Transformation Process

APHIS considered the potential for the transformation process to cause or aggravate disease symptoms in Event VCO-Ø1981-5 corn or other plants or to cause the production of plant pathogens. Wild type *Agrobacterium tumefaciens* carries a tumor-inducing (Ti) plasmid that can be transferred to broadleaf plants and cause crown gall disease. *Agrobacterium tumefaciens* strain LBA4404 contains a disarmed Ti plasmid that is incapable of inducing tumor formation due to the deletion of the phytohormone genes originally present in the *Agrobacterium* plasmid (Koncz and Schell 1986). Event VCO-Ø1981-5 corn was produced by *Agrobacterium tumefaciens*-mediated transformation with plasmid pAG3541 (Genective 2011, p.19). This transformation process did not lead to crown gall disease in Event VCO-Ø1981-5. Instead, in the T-DNA region a maize-optimized *epsps grg23ace5* gene and regulatory components necessary for its expression in the corn genome were introduced. *Agrobacterium*-mediated plant transformation has been used widely for decades, has not been implicated in causing plant disease, and is highly unlikely to pose a plant pest risk.

Introduced genes and their genetic elements

APHIS reviewed Southern blot analysis data that demonstrates that Event VCO-Ø1981-5 corn regenerated from the transformation event contains a single copy of the gene cassette (Genective 2011, Appendix 3, pp. 134-135). No region from the backbone of plasmid pAG3541 was inserted (Genective 2011, Appendix 3, pp. 140-142). The stability of the inserted DNA was also evaluated over four generations by using molecular and phenotypic methodologies (Genective 2011, Appendix 3, p. 139). Plant phenotypes were evaluated to ensure stability of the traits during the plant breeding process by spraying plants with the herbicide glyphosate. Mendelian segregation of the inserted genes was analyzed using Chi-square analysis over 4 generations (Genective 2011, p. 28-29). Each

generation of plants was treated with glyphosate to eliminate those plants that were not herbicide-tolerant, reflecting a lack of inheritance of the *epsps gre23ace5* gene. Table 5 (p. 29) of the Petition shows the expected and observed segregation for the four generations tested. There was no significant deviation from the expected 1:1 ratio.

Event VCO-Ø1981-5 corn expresses a modified 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS). The native *epsps gre23ace5 gene* was isolated from *Arthrobacter globiformis,* a common soil-inhabiting bacterium. The native EPSPS GRG23 protein was modified for optimal expression in corn, in part, by changing ten amino acids to produce EPSPS ACE5. EPSPS ACE5 is 97.6% identical and 98.5% homologous at the amino level to the originally isolated EPSPS GRG23 protein. EPSPS ACE5 encodes a 44.3 kDa (kilo Daltons) protein consisting of a single polypeptide of 402 amino acids. The family of EPSPS proteins is ubiquitous in plants and microorganisms. EPSP synthase is a critical enzyme in the shikimate pathway mediating the biosynthesis of aromatic compounds in plants, bacteria and fungi. This pathway is absent in mammals, fish, birds, reptiles and insects (Alibhai and Stallings 2001). EPSPS proteins have been extensively studied (ILSI 2010) and not found to cause disease or the production of infectious agents in plants. A safety assessment of EPSPS ACE5 protein was submitted by Athenix to FDA in 2009 for the Early Food Safety Evaluation. FDA completed its review with no further questions in 2010 (US-FDA 2010).

The terminator for the *epsps gre23ace5* gene is derived from cauliflower mosaic virus (CaMV) which is a plant viral pathogen (which causes disease primarily in cruciferous plants). The CaMV 35S terminator sequence is non-coding, contains regulatory sequences of known function and does not cause disease symptoms in plants nor encode for an infectious agent.

Genective performed sequencing analysis around the flanking regions of the insertion in event VCO-Ø1981-5 and found:

- A 21 base pairs (bp) deletion in the corn genome was identified. Bioinformatics analysis identified 12 potential open reading frames (ORFs). Regarding allergenicity potential, no ORFs share any amino acid sequence similarities or homology compared with known allergens. These ORF proteins were also analyzed against publically available databases and none showed significant homology with proteins known to be toxins. In addition, the sequencing analysis found no “start” codons upstream. It is highly unlikely for these ORFs to generate any translatable mRNA. Therefore, the potential for any of the expressed proteins to be food allergens or toxins is minimal.

- The insertion of the T-DNA was integrated at maize chromosome1 and has occurred at the 5’ untranslated region of endogenous Acanthoscurrin and could potentially code for an Acanthoscurrin-homolog protein. It has been noted in the literature that Acanthoscurrin is a linear cationic antimicrobial glycine-rich protein (GRP) (Lorenzini et al. 2003). GRPs with a variety of functions have been found in different organisms (Remuzgo et al. 2005). Acanthoscurrins isolated from *Acanthoscurria gomesiana* (tarantula spider) have been noted in the

literature when tested against microorganisms to display activity against the gram-negative bacteria *E. coli* and against the fungus *Candida albicans* (Lorenzini et al. 2003). However, Acanthoscurrins isolated from *Acanthoscurria gomesiana* (tarantula spider) have no structural similarities with already known glycine-rich antimicrobial peptides from animals and plants (Lorenzini et al. 2003). There are no data or evidence that Acanthoscurrins in the Event VCO-Ø1981-5 corn are involved in resistance to bacterial or fungal disease.

Event VCO-Ø1981-5 corn was field tested in 17 U.S. field sites in 2009 to collect agronomic data. Genective evaluated whether plant-disease or plant-insect interactions, or plant response to abiotic stressors of event VCO-Ø1981-5 corn were altered compared to the negative segregant controls. Across all sites, a total of 9 insect categories, 6 disease categories and 2 abiotic stressors were evaluated (Genective 2011, pp.51-52). There were no qualitative differences observed in Event VCO-Ø1981-5 corn compared to its negative segregants. Since no significant differences were observed across sites, it was concluded that there is no apparent potential for significant impact on disease and pest susceptibilities.

Compositional Analysis

Compositional analyses were conducted to assess whether the composition and nutrient levels in grain and forage derived from Event VCO-Ø1981-5 corn were comparable to the null segregant, which has background genetics very similar to Event VCO-Ø1981-5 corn but without the *epsps grg23ace5* gene cassette. Compositional comparisons between Event VCO-Ø1981-5 corn and negative segregant controls were performed using the principles and analytes outlined in the OECD consensus documents for composition (OECD, 2002) of new varieties of corn. Three additional conventional corn varieties selected by Genective, which were commonly planted commercially in their adapted geographic region and were currently in the marketplace, were also included in the analysis to establish a range of natural variability for each analyte. For each compositional experiment, a 99% tolerance interval was calculated. This interval is expected to contain, with 95% confidence, 99% of the values obtained from the population of commercial reference varieties. By comparison to the 99% tolerance interval, any statistically significant differences between Event VCO-Ø1981-5 corn and the comparators may be put into perspective, and can be assessed for biological relevance in the context of the natural variability in corn. In addition, for the compositional assessment, publicly available information was obtained by Genective from International Life Science Institute (ILSI) Crop Composition Database (ILSI 2006) for corn as "Published Range." Event VCO-Ø1981-5 corn analyte ranges that fell within the tolerance interval and/or published range for that analyte were considered to be within the range of normal variability of commercial corn hybrids.

The Genective compositional analyses of Event VCO-Ø1981-5 corn and the genetically very similar negative segregants were based on forage and grain harvested from 2009 at five field locations in three states (Genective 2011, p. 53). Compositional analyses of grain samples included moisture, protein, fat, acid detergent fiber, neutral detergent fiber, crude fiber, total dietary fiber, starch, ash, and carbohydrates. Compositional analyses of

forage samples included protein, fat, acid detergent fiber, neutral detergent fiber, crude fiber, ash, calcium, and phosphorus and carbohydrates.

Genective also measured the concentrations of the expressed protein-EPSPS ACE5 in various growth stages (Genective 2011, Table 8 and 9, p. 36-37). EPSPS ACE5 protein is homologous to EPSPS proteins naturally present in plants (e.g., soybean and corn) and fungal and microbial sources, all of which have a history of safe human consumption (Harrison et al., 1996). The EPSPS ACE5 protein expressed in Event VCO-Ø1981-5 corn is 97.6% identical and 98.5% homologous at the amino acid level to its originally isolated native enzyme EPSPS GRG23 from *Arthrobacter globiformis*. *Arthrobacter* species are found ubiquitously in soil. Athenix Corporation provided the FDA with information on the identity, function, and characterization of the gene for Event VCO-Ø1981-5 corn, including expression of the gene products in an Early Food Safety Evaluation of new proteins in new plant varieties. The FDA completed its review of Athenix' submittal and published a response with "no further questions" in October 2010 (US-FDA 2010).

Overall, a comprehensive evaluation of Event VCO-Ø1981-5 corn and the controls showed no biologically meaningful differences for grain and forage compositions for either major nutrients (Genective 2011, pp.53-65) or key anti-nutrients in corn grain (Genective 2011, p.64). The few detected differences were either exceedingly small in magnitude or the mean component values of Event VCO-Ø1981-5 corn and the control were within the 99% tolerance interval. Therefore, based on the data presented by Genective on forage and grain, it is reasonable to conclude that the foods and feeds derived from Event VCO-Ø1981-5 corn can be considered compositionally and nutritionally equivalent to those derived from conventional corn.

Agronomic Properties

Genective conducted agronomic evaluations on Event VCO-Ø1981-5 corn and conventional and /or control corn lines. These evaluations are used to determine whether Event VCO-Ø1981-5 corn is agronomically comparable to conventional corn. They also provide reasonable scientific measures as to whether Event VCO-Ø1981-5 corn has plant pest potential. These agronomic assessments included seven (7) parameters of vegetative characteristics (plant height, ear height, final stand count, stalk lodging, root lodging, grain moisture and grain weight), 5 parameters of reproductive characteristics (days to 50% pollen shed, days to 50% silking, dropped ears, barrenness and yield), seed emergence and dormancy, and several biotic and abiotic stress characteristics (disease incidence and insect damage) (Genective 2011, pp. 46-53). The agronomic data showed no significant differences between Event VCO-Ø1981-5 corn and control corn lines. These data support Genective's claim that Event VCO-Ø1981-5 corn is agronomically comparable to conventional corn except for the intended traits and that Event VCO-Ø1981-5 corn does not possess characteristics that constitute plant pest risk compared to conventional corn.

Event VCO-Ø1981-5 corn and its control lines have been field tested in the U.S. and its territories since 2007 and outside of the U.S. (Spain, Czech Republic, Romania, Slovak Republic and Canada) since 2009 (Genective 2011, pp. 42-43). The principal investigator

of the trials surveyed the fields for naturally occurring insects, diseases and any unexpected differences between Event VCO-Ø1981-5 corn and control lines are summarized in Table 19-21 of the Petition. The data of phenotypic and ecological assessments of Event VCO-Ø1981-5 corn provided by Genective collectively support the petitioner's claim that Event VCO-Ø1981-5 corn does not exhibit any meaningful differences when compared to its non-transgenic negative segregant counterparts in regards to naturally-occurring insect or disease infestations.

The data (transformation method, introduced genes and their genetic elements, and compositional analysis and agronomic properties) cited by Genective and reviewed by APHIS indicate that Event VCO-Ø1981-5 corn is not biologically different from non-transgenic conventional corn lines (with the exceptions of intended introduced genetic constructs and trait) and Event VCO-Ø1981-5 corn is no more susceptible to pests and diseases compared to conventional corn. The introduced genetic constructs and trait (tolerance to glyphosate herbicide) are not expected to alter disease and pest susceptibilities.

D. Potential for Enhanced Weediness of Event VCO-Ø1981-5 Corn

In the U.S., corn is not listed as a weed (Crockett 1977; Muenscher 1980), nor is it present on the Federal Noxious Weed List (7 CFR part 360.200). Corn is grown throughout the world without any report that it is a serious weed or that it forms persistent feral populations (Gould 1968). Like many domesticated crops, corn seed from a previous year's crop can overwinter and germinate the following year. Manual or chemical measures are often applied to remove these volunteers, but the plants that are not removed do not typically result in feral populations in following years because corn is incapable of sustained reproduction outside of domestic cultivation and corn is non-invasive in natural habitats (Gould 1968). Corn possesses few of the characteristics of those plants that are notably successful as weeds (Baker 1965; Keeler 1989). Compared to other corn varieties, Event VCO-Ø1981-5 corn has improved fitness in the presence of glyphosate herbicide. But, there are many available options for the control of Event VCO-Ø1981-5 corn if unwanted plants might be growing in the field.

APHIS assessed whether Event VCO-Ø1981-5 corn is any more likely to become a weed than the non-transgenic comparator corn line or other corn varieties currently under cultivation. The assessment encompasses a thorough consideration of the basic biology of corn and an evaluation of the unique characteristics of Event VCO-Ø1981-5 corn. Genective has been conducting agronomic evaluations in both laboratory experiments and field trials.

The germination and dormancy evaluations were conducted under laboratory conditions. Seed dormancy is an important characteristic that is often associated with plants that are considered weeds (Anderson 1996). Although dormancy is not associated with modern corn cultivars, corn seed dormancy tests can be used to determine whether Event VCO-Ø1981-5 corn is agronomically comparable to conventional corn and determine whether

[2] http://www.aphis.usda.gov/plant_health/plant_pest_info/weeds/downloads/weedlist-2010doc.pdf

Event VCO-Ø1981-5 corn is more likely to pose a plant pest risk when compared to conventional corn.

Standardized germination assays of the Association of Official Seed Analysts (AOSA 2007) are often used as a baseline to measure seed germination potential. Under warm germination, Event VCO-Ø1981-5 corn and its non-transgenic controls have an equivalent germination rate of 99.6%. Under cold germination, Event VCO-Ø1981-5 corn has a 97% germination rate vs. 93% for non-transgenic controls (Genective 2011, pp. 52-53). The results (Genective 2011, Table 22, p.53) support the conclusion that Event VCO-Ø1981-5 corn is comparable to conventional corn with minor differences and Event VCO-Ø1981-5 corn does not exhibit characteristics that would cause it to be weedier than the parental lines or conventional varieties.

A total of 13 different agronomic phenotypic characteristics, as well as observations for plant responses to plant-disease and plant-insect interactions were evaluated by Genective (Genective 2011, Tables16-21, pp.48-52) at 17 U.S. locations, 2 Canadian locations and more than 20 locations in Europe from 2007 through 2011 (Genective 2011, Tables12-13, p.42-43). At these locations, the range of values for agronomic parameters is within the range of values expected for non-GE commercial corn hybrids. Agronomic characteristics evaluated (Genective 2011, Table 16-18, pp.48-50) include: emergence, plant height, final stand count, stalk lodging, root lodging, grain moisture, grain weight, days to 50% pollen shed, days to 50% silking, dropped ears, barrenness, yield, disease incidence and insect damage. No differences in undesirable phenotypic characteristics, including weediness traits, were identified. The mean values of these agronomic traits observed for Event VCO-Ø1981-5 corn fall within the range of values observed for the commercial corn varieties. Field trial data (Genective 2011, Appendix 1, pp. 85-114) indicates that Event VCO-Ø1981-5 corn does not exhibit characteristics that would cause it to be weedier than the controls and non-GE conventional corn.

In addition, agronomic field observation data for Event VCO-Ø1981-5 corn and the non-transgenic counterparts shows no significant differences in disease incidence and insect damage. The introduced EPSPS protein provides tolerance to the herbicide glyphosate for Event VCO-Ø1981-5 corn and this trait occurs in a total of seven commercially grown plant species given non-regulated status by USDA-APHIS since 1994 (e.g., Petition 93-258-01p, Petition 95-045-01p, Petition 97-099-01p, Petition 98-173-01p, Petition 98-216-01p, etc. found here: http://www.aphis.usda.gov/biotechnology/not_reg.html). The plant species that have been engineered to express the protein EPSPS are: *Beta vulgaris* L. (sugarbeet), *Brassica napus* L., *Brassica rapa* L., *Glycine max* L. (soybean), *Gossypium hirsutum* L. (cotton), *Medicago sativa* L. (alfalfa) and *Zea mays* L. (maize). These GE plant species also have some potential to "volunteer" as weeds in following growing seasons (OECD 2009; OECD 2003). The available data indicate there is no linkage between expression of EPSPS proteins and any increased survival or overwintering capacity that would alter the prevalence of volunteer plants in subsequent growing seasons. These changes will not necessitate a major departure from well-established and broadly used agricultural protocols. The first glyphosate tolerant corn was given nonregulated status in 1997 and was on the market soon after. Based on 2008 survey data, glyphosate tolerant corn, soybean and cotton accounted for approximately

three quarter of the total commercially grown corn, cotton and soybean planted in the US (Nandula 2011). After many years of commercialization of glyphosate tolerant corn, soybean and cotton, these plants have not exhibited characteristics that would indicate increased weediness. There is no reason to expect that Event VCO-Ø1981-5 corn would result in increased weediness, since Event VCO-Ø1981-5 corn contains a version of EPSPS protein that provides similar glyphosate tolerance as these other varieties. Based on agronomic and compositional data showing that EPSPS does not have a significant impact on agronomic or compositional traits (including those that are related to weediness), there is no evidence to date that expression of EPSPS protein has resulted in any altered potential for weediness in these GE plants. The above considerations, together with the fact that the novel trait has no intended effect on weediness, leads USDA-APHIS to conclude that Event VCO-Ø1981-5 corn has no altered weediness potential compared to current commercialized glyphosate tolerant varieties. Additionally, field evaluation of phenotypic and agronomic characteristics showed no differences relative to its comparators and supports the conclusion that Event VCO-Ø1981-5 corn is not likely to have increased weediness compared to conventional corn varieties.

E. Potential to Impacts the Weediness of Other Plants with which It Can Interbreed

APHIS evaluated the potential for gene introgression to occur from Event VCO-Ø1981-5 corn to sexually compatible wild relatives and considered whether such introgression would result in increased weediness. Introgression is a process whereby gene(s) successfully incorporate into the genome of a recipient plant.

Corn belongs to the grass family, *Poaceae*. The genus *Zea* has five species: *diploperennis* HH, *perennis, luxurians, mays,* and *nicaraguensis*(OGTR 2008). *Zea mays* is further divided into four subspecies: *huehuetenangensis, mexicana, parviglumis,* and *mays*. The first three subspecies are teosintes. *Z. mays* ssp *mays* is the only cultivated species and has been domesticated for its current use by selection of key agronomic characters, such as a non-shattering rachis, grain yield, and resistance to pests (Wozniak 2002). *Zea mays* ssp *mays* occurs only where it is cultivated in the U.S. Occasionally it is found in abandoned fields or on roadsides. The closest wild relatives of corn are the teosintes (wild *Zea* spp.) (Ellstrand et al. 2007) which are sexually compatible with *Zea mays*. All teosinte members can be crossed with cultivated corn to produce fertile first generation hybrids (Doebley1990; Wilkes 1967). However corn teosinte hybrids exhibit low fitness and have little impact on gene introgression in subsequent generations (Galinat 1988). Additionally, teosintes are normally confined to the tropical and subtropical regions of Mexico, Guatemala, and Nicaragua. A fairly rare, sparsely dispersed feral population of teosinte has been reported in Florida (USDA PLANTS Database[3], accessed 4/18/2012).

Tripsacum is a genus of grass in the Poaceae family. Although it is difficult, *Tripsacum* can be successfully hand crossed with corn to form hybrids. However these hybrids have a high degree of sterility (Doebley 1990; Wilkes 1967) and are generally unstable because of differences in chromosome number and lack of pairing between chromosomes

[3] http://plants.usda.gov/java/county?state_name=Florida&statefips=12&symbol=ZEME

(Eubanks 1997). First generation hybrids are much less fit for survival and dissemination in the wild and typically show reduced reproductive capacity. Furthermore, gene flow from corn to *Tripsacum* is virtually impossible because of several factors including distribution, genetic incompatibility, temporal separation of flowering time, etc. (Galinat 1988). These distinctions between related species directly affect the ability of cultivated corn to interbreed with wild relatives. Modern corn is highly domesticated and requires significant human intervention to grow and reproduce. As with all domesticated corn, the likelihood that Event VCO-Ø1981-5 corn would reproduce and sustain populations outside of cultivation is extremely small.

Corn is predominantly an outcrossing plant species. The rate of self-pollination is 5% (Sleper and Poehlman, 2006). The short viability period of pollen grains limits significant outcrossing. Since Event VCO-Ø1981-5 corn does not exhibit characteristics that can cause it to be any weedier than other cultivated corn, its potential for gene introgression into teosinte is not expected to be any different from that of other cultivated corn varieties.

None of the sexually compatible relatives of corn in the U.S. are considered to be weeds in the U.S. (Holm et al. 1979); and, as discussed in the section above, the unlikely acquisition of an herbicide tolerance gene would not be expected to transform them into weeds. Therefore, USDA has concluded that adverse consequences of gene flow from Event VCO-Ø1981-5 corn to wild or weedy species in the U.S. are highly unlikely.

F. Potential Impacts on Non-target Organisms, Including Those Beneficial to Agriculture

APHIS evaluated the potential for Event VCO-Ø1981-5 corn to have damaging or toxic effects directly or indirectly on non-target organisms. The non-target organisms considered were representatives of the exposed species in the agricultural environment. As discussed earlier, Event VCO-Ø1981-5 corn is similar (by compositional analysis) to unmodified control corn varieties except for the intended changes in herbicide resistance, associated with the production of EPSPS ACE5 protein in the plant.

Event VCO-Ø1981-5 corn expresses the EPSPS ACE5 protein which provides resistance to the herbicide glyphosate. It was derived from EPSPS GRG23 protein isolated from common soil bacteria *Arthrobacter globiformis.* The EPSPS ACE5 protein was optimized for incorporation into corn by changing ten amino acids from the EPSPS GRG23 protein (Schouten etal. 2011; Genective 2011, page 31). This codon modification makes EPSPS ACE 5 closely match the native maize EPSPS and did not alter its enzymatic profile (Genective 2011, page 33-34). The EPSPS ACE5 is still 97.6% identical to the originally isolated native EPSPS GRG23.

For EPSPS ACE5 protein, a bioinformatics analysis was performed by Athenix which determined that the amino acid sequence of the EPSPS ACE5 protein exhibits no significant homology to known or suspected allergens and toxins (Athenix 2009). Further, the resistance to enzymatic pepsin digestion of EPSPS ACE5 protein was analyzed (Thomas et al. 2004). The results indicate that EPSPS ACE5 is readily digested

in mammalian digestive systems and rapidly degraded (Athenix, 1999; Genective 2011, p.40). Rapid degradation of the full-length EPSPS ACE5 protein makes it highly unlikely that EPSPS protein would be absorbed by epithelial cells of the small intestine in a biologically-active form. Acute oral toxicity studies in mice show that EPSPS ACE5 has no adverse effect on acutely gavaged mice (Athenix 1999). Doses of 1783 mg/kg bodyweight were administered to mice, and there were no signs of toxicity in any of the groups of mice tested over a period of 14 days. Acute toxicity studies in mice show the EPSPS ACE5 protein has no toxicity even at doses much higher than mice could encounter due to exposure to GE plants expressing the EPSPS protein. Based on this study, EPSPS ACE5 protein did not show any evidence of toxicity.

APHIS considered the similarity in structure and function of the CP4 EPSPS found in a number of Roundup Ready® crops with nonregulated status to EPSPS ACE5, and other EPSPS proteins endogenous to plants and present throughout the environment. The EPSPS ACE5 protein in Event VCO-Ø1981-5 corn is highly homologous to the family of EPSPS proteins and the CP4 EPSPS protein that has a long history of safe use in these plants. In 2011, glyphosate-resistant varieties were grown on approximately 93 percent of soybean acres, 78 percent of upland cotton acres, and 70 percent of corn acres in the United States (USDA-ERS 2011). The CP4 EPSPS proteins have been commercialized since 1994 and are present in crops which are grown on millions of acres in the U.S. every year. At present, APHIS is not aware of any identified significant adverse effects of EPSPS proteins on the abundance of non-target organisms in the field. The family of EPSPS proteins present in a variety of plants has been subject to extensive human consumption without evidence of health concerns.

The Environmental Protection Agency (EPA) also has previously reviewed the safety of the EPSPS protein and has established a tolerance exemption for the protein (US-EPA 1997). The exemption was based on a safety assessment. CP4 EPSPS and other versions of EPSPS proteins were evaluated for safety by FDA concluding that “no further questions” were needed. The food and feed products containing EPSPS proteins are apparently as safe as corn currently on the market for human and animal consumption. In 2009, Athenix submitted EPSPS ACE5 protein for the Early Food Safety Evaluation. FDA completed the review with no further questions in 2010 (US-FDA 2010). To assess unintended effects, APHIS analyzed data submitted by the developer to determine if there were changes to phenotype, germination, vegetative growth, reproductive parameters and response to biotic stressors (insect and disease stress) associated with Event VCO-Ø1981-5 corn in comparison to the various control lines (nontransgenic). Data presented in the Petition (Genective 2011, page 43-55) indicates that the ecological interactions between Event VCO-Ø1981-5 corn and the control lines were similar.

Considering all the data noted from field observations, the lack of toxicity in an acute mouse feeding study, lack of homology to toxins and allergens, widespread presence in the environment and known safety information on EPSPS proteins, APHIS concludes that Event VCO-Ø1981-5 corn is unlikely to have any adverse impacts to nontarget organisms and beneficial organisms.

G. Potential Impacts from Transfer of Genetic Information to Organism with which Event VCO-Ø1981-5 Corn Cannot Interbreed

APHIS examined the potential for the new genetic material inserted into Event VCO-Ø1981-5 corn 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. Horizontal gene transfer and expression of DNA from a plant species to other species is highly unlikely to occur based on the following reasons.

The horizontal gene transfer (HGT) between unrelated organisms is one of the most intensively studied fields of science. Horizontal gene transfer and expression of DNA from a plant species to bacteria or animal species is unlikely to occur. A number of points support this conclusion:

- 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 et al. 2002). There is no evidence that these organisms contain genes derived from plants. Also, *Agrobacterium tumefaciens* or *Arthrobacter globiformis* species are generally common in soil and therefore various *epsps* genes have been available for long periods of time for horizontal transfer from *Agrobacterium tumefaciens* or *Arthrobacter globiformis* to plants or other soil microorganisms and decaying plant material. Therefore the likelihood of any impact or new horizontal gene transfer that is not already capable of taking place in the soil is extremely unlikely.

- No evidence has been identified for any mechanism by which maize genes could be transferred to humans or animals, or any evidence that such gene transfer has occurred for any plant species during evolutionary history, despite animals and humans eating large quantities of plant DNA. 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).

- 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.

- 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 extremely unlikely (US-FDA 1998).

- APHIS also considered whether horizontal transfer of DNA from Event VCO-Ø1981-5 corn to plant viruses was likely to occur and would lead to the creation

or selection of a more virulent plant pathogen through recombination with other plant viruses. This issue has been considered before by other science review panels and government regulatory bodies (Keese 2008). Although sequences of the Cauliflower Mosaic Virus are contained within Event VCO-Ø1981-5 corn, those sequences are limited to the regulatory elements. Regulatory elements such as promoters and terminators have not been implicated in viral recombination.

Finally, under natural conditions; no transfer of an intact functional gene has been demonstrated to date (Miki and McHugh, 2004).Therefore, APHIS concludes that horizontal gene transfer is unlikely to occur and thus poses no plant pest risk.

H. Potential Changes to Agriculture or Cultivation Practices

APHIS considered potential impacts associated with cultivation of Event VCO-Ø1981-5 corn on current agricultural practices in corn and other crops. Genective has provided data which indicate that Event VCO-Ø1981-5 corn is comparable to conventional corn in phenotypic, ecological and compositional characteristics (Genective 2011). Event VCO-Ø1981-5 corn, a glyphosate tolerant variety, is expected to replace some presently available commercial glyphosate tolerant corn varieties without affecting the overall total corn acreage or glyphosate tolerant corn acreage. The availability of more herbicide tolerant corn products will likely increase grower choice and price competition.

Compared to currently-available glyphosate tolerant corn products containing EPSPS, no increased use of herbicides from the use of Event VCO-Ø1981-5 corn is expected. In 2010, glyphosate-resistant varieties were grown on approximately 93 percent of soybean acres, 78 percent of upland cotton acres, and 70 percent of corn acres in the United States. As these varieties were adopted, farmers generally used glyphosate as an herbicide and weed-management tactic. In general, glyphosate is less toxic to humans than other common herbicides and not as likely to persist in the environment as many of the herbicides it replaces (IPCS 1994; USDA-ERS 2006). APHIS does not foresee any increased glyphosate use by the addition of Event VCO-Ø1981-5 corn to the market.

Continuous use of one herbicide to control weeds will select for weed resistance. In general, weed problems in fields of GE glyphosate-resistant crops will become more common as weeds evolve resistance to glyphosate or weeds less susceptible to glyphosate become established in areas treated exclusively with the same herbicide. A number of new genetically engineered, herbicide-resistant corn varieties are currently under development and may provide growers with other herbicides and weed management options when fully commercialized. Growers need to consider other effective weed-management tools or use the alternative herbicides with different modes of actions. Such practices should be encouraged through collaborative efforts by federal and state government agencies, private-sector technology developers, universities, and farmer organizations to develop cost-effective resistant-management programs and practices that preserve effective weed control in herbicide-resistant crops (Ervin 2010).

Overall, APHIS concludes that agricultural or cultivation practices are not expected to change and thus no changes in plant pest risks are expected due to such practices.

I. Conclusion

APHIS has prepared the plant pest risk assessment in order to determine if Event VCO-Ø1981-5 corn is unlikely to pose a plant pest risk. Based on the information provided by the applicant and the lack of plant pest risk from the inserted genetic material, weedy characteristics, atypical responses to disease, insects or plant pests in the field, effects on non-targets or beneficial organisms in the agro-ecosystem, changes to agricultural practices, and horizontal gene transfer, APHIS has concluded that Event VCO-Ø1981-5 corn is highly unlikely to pose a plant pest risk.

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