

## **Plant Pest Risk Assessment for DAS-40278-9 Corn**

Dow AgroSciences LLC (referred to hereafter as DAS) has petitioned APHIS (APHIS number 09-233-01p, referenced as DAS 2010) for a determination that genetically engineered (GE) corn (*Zea mays*) event DAS-40278-9 is unlikely to pose a plant pest risk and, therefore, should no longer be a regulated article under regulations at 7 Code of Regulations (CFR) part 340. APHIS administers 7 CFR part 340 under the authority of the plant pest provisions of the Plant Protection Act of 2000<sup>1</sup>. This plant pest risk assessment was conducted to determine whether event DAS-40278-9 corn is unlikely to pose a plant pest risk.

### **History of Development of DAS-40278-9 Herbicide-Tolerant<sup>2</sup> Corn**

DAS-40278-9 corn is a GE corn line that has been provided increased tolerance to treatment with phenoxy auxin herbicides and resistance to aryloxyphenoxypropionate (AOPP) acetyl coenzyme A carboxylase (ACCase) inhibitor (“fop”) herbicides. The introduced genetic material (DNA) results in the production of an aryloxyalkanoate dioxygenase (AAD-1) enzyme that inactivates herbicides of the aryloxyalkanoate family, including phenoxy auxins and AOPP ACCase inhibitors. If a determination of nonregulated status is reached, DAS-40278-9 corn would be the first commercially available corn variety with both improved tolerance to phenoxy auxin herbicides and resistance to “fop” herbicides.

The most well-known and widely-used phenoxy auxin herbicide is 2,4-dichlorophenoxyacetic acid (2,4-D) which has been used for many decades as a pre-plant or post-emergent herbicide to control broadleaf (dicot) weeds in corn fields. 2,4-D is also labeled for use for many other weed control applications including with other crops, on pastures and rangeland, and by both commercial and residential entities for weed control in turf and lawns. The mode of action and the relative biochemical selectivity of 2,4-D for broadleaf plants is unclear, but it is believed to function as a plant growth regulator (auxin hormone) mimic which causes abnormal cell division and growth leading to plant injury and death (DAS 2010, pages 154-155; EPA 2005). Though it is not a broadleaf plant, corn can suffer injury from 2,4-D depending on the growth stage and method of application (DAS 2010, pages 103-105).

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<sup>1</sup> 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.”

<sup>2</sup> The applicant has described DAS-40278-9 corn as “herbicide tolerant” and historically APHIS has also referred to GE plants with diminished herbicide sensitivity as “herbicide tolerant”. However, the phenotype would fall under the Weed Science Society of America’s definition of “herbicide resistance” since DAS-40278-9 has an inherited ability to survive and reproduce following exposure to a dose of herbicide normally lethal to the wild type variety (WSSA 1998). By the WSSA definition, “resistance [to an herbicide] may be naturally occurring or induced by such techniques as genetic engineering or selection of variants produced by tissue culture or mutagenesis.” Herbicide tolerance, by the WSSA definition, only applies to plant species with an “inherent ability” to survive and reproduce after herbicide treatment.

AOPP ACCase inhibitors, or “fop” herbicides, are post-emergent graminicides, meaning that the herbicides selectively control emerged grass (Poaceae) weeds. The selectivity of the “fops” is based on an increased sensitivity of grass species to ACCases compared to other plant species (Devine 1997; Konishi and Sasaki 1994). The consequence of treatment with an ACCase inhibitor herbicide is to block production of fatty acid biosynthesis in sensitive plants. Corn, a plant in the Poaceae family, along with other related cereal plant varieties, is conventionally sensitive to treatment with “fop” herbicides, and these herbicides have not been traditionally labeled for weed control in corn fields (WSSA 2007). While some cereal crop plants have developed some tolerance to “fop” treatment due to improved herbicide detoxification (DAS 2010, pages 155-156; Devine 1997), and corn varieties with resistance to “fop” treatment have been isolated using mutation screening (Marshall et al. 1992), if a determination of nonregulated status is reached, DAS-40278-9 corn may be the first corn variety with resistance to these herbicides to become widely commercially available.

If a determination of nonregulated status is reached, DAS-40278-9 would provide corn growers with additional options for the post-emergent control of both broadleaf and grass weeds. This corn product would also provide a potential remedy to the increased incidence of glyphosate and acetolactate synthase (ALS) inhibitor resistant weeds. The petitioner has studied various agronomic and compositional components of DAS-40278-9 corn since 2006 through confined field tests, and, in accordance with 7 CFR part 340.6(c), has presented data in the submitted petition for a determination that DAS-40278-9 corn should no longer be regulated under 7 CFR part 340. Additionally, per correspondence with the petitioner, APHIS is aware that DAS-40278-9 corn was submitted for a Consultation of Bioengineered Foods to the FDA on October 1, 2009 under the FDA’s Statement of Policy: Foods Derived from New Plant Varieties (Federal Register Volume 57 No. 104, 1992, page 22984). The petitioner also communicated (DAS 2010, page 18, and personal communication to APHIS on July 11 and 22, 2010) that label change requests would be made by the fourth quarter of 2010 to the EPA for the use of 2,4-D and quizalofop herbicides for weed management in fields with DAS-40278-9 corn.

In 2009, approximately 68% of corn planted in the United States possessed resistance to an herbicide that was conferred through biotechnology (USDA NASS 2009). The primary herbicide resistance trait in use has been glyphosate resistance, and the adoption of this trait in other major crops, such as cotton and soybean, is even higher. Weed resistance to herbicides is a concern in agricultural production and the wide-spread adoption of herbicide resistant crops, especially GE-derived glyphosate-resistant crops, has dramatically changed the approach that farmers take to avoid yield losses from weeds (Duke and Powles 2009; Gianessi 2008). Glyphosate-resistant crops have become widely adopted since their introduction in the mid-late 1990s for several reasons. Glyphosate works non-selectively on a wide range of plant species, is a relatively low-cost herbicide, allows ‘no-till’ farming practices, and has minimal animal toxicological and environmental impact. However, increased selection pressure resulting from the wide-spread adoption of glyphosate-resistant crops, along with the reductions in the use of other herbicides and weed management practices, has resulted in both weed population shifts and growing numbers of glyphosate resistance among some weed populations (Duke and Powles 2009; Owen 2008). In order to combat this trend, and to avoid decreased crop yields that result from weed competition, growers must continue to adapt their weed management strategies. If

DAS-40278-9 corn becomes available to them, corn farmers who choose to grow it will have added options for weed control. The use of DAS-40278-9 corn could allow for improved corn crop yields when it is grown in the vicinity of weeds resistant to glyphosate, particularly with anticipated labeling changes allowing the expanded application of 2,4-D at a critical time period for controlling weeds that compete with corn during the first 3-5 weeks before the corn reaches 6-8 inches in height and label changes allowing over-the-top post-emergent use of quizalofop (DAS 2010, pages 108 and 113). This practice of using herbicides with alternative modes of action could also potentially diminish the populations of those glyphosate-resistant weeds and reduce the likelihood of the development of new herbicide-resistant weed populations (DAS 2010, pages 16-18 and Appendix 6; Duke and Powles 2008, 2009; Dill et al. 2008; Owen 2008).

The herbicide 2,4-D was introduced more than 60 years ago and is used throughout the world for the treatment of broadleaf weeds. In 2002, 2,4-D was ranked as the third most used herbicide by active ingredient in the U.S. for all purposes (~ 40 million pounds), behind glyphosate (~102 million pounds) and atrazine (~77 million pounds) (Gianessi and Reigner 2006). That same report found that the use of 2,4-D remained relatively steady from 1992 to 2002, whereas glyphosate usage increased more than 5-fold over the same time period. This increase in glyphosate use is attributable to the introduction and wide-spread adoption of GE glyphosate resistant crop species (e.g., soybeans, corn, cotton, canola) and rising adoption of no- or reduced-till farming practices that typically accompanies the use of glyphosate. The report also indicated that overall herbicide use on corn decreased during that same 10 year time frame (from ~213 million pounds in 1992 to ~159 million pounds in 2002), as farmers increasingly favored the use of GE crops which allowed for fewer types and a smaller overall amount of herbicide to be used.

The EPA did an analysis of herbicide usage in corn fields for 2004 that also showed that the use of glyphosate increased dramatically from 1987 to 2001 whereas 2,4-D usage remained essentially unchanged during that time (Kiely 2004). In 2005, herbicides were applied to 97 percent of corn acreage planted in 19 states representing 93 percent of all corn planted in the United States. The herbicide most widely used on corn was atrazine (66 percent, ~57 million pounds applied), glyphosate was second (31 percent, ~23 million pounds), followed by s-metolachlor and acetochlor (both at 23 percent, <24 million pounds and <30 million pounds, respectively). By comparison, 2,4-D was applied on less than 8 percent of 2005 corn acreage (~2 million pounds applied) (USDA NASS 2006). The highest recorded use of 2,4-D is 14 percent of U.S. corn acres in 1994 (<http://www.pestmanagement.info/nass/index.html>, Accessed 3/2010). These data demonstrate that there is a history of successful and effective use of 2,4-D as an herbicide, both generally and specifically on corn crops, to eliminate weed species. Because corn is not a broadleaf plant, it already has tolerance to 2,4-D, and the addition of a trait for increased tolerance to 2,4-D is not anticipated by the petitioner to greatly change the historical levels of herbicide use at the field level. Instead, DAS-40278-9 corn will give growers the option to apply 2,4-D during different growing windows, using different application methods and herbicide mixes (DAS 2010, pages 113-115). The expectation from the petitioner is that this added trait will help to minimize the emergence of herbicide resistant weeds because based on field efficacy studies (DAS 2010, pages 103-105) the DAS-40278-9 corn plants are less susceptible to injury associated with proposed application rates of 2,4-D applied either at the pre-emergent stage or later post emergent stages, and thus will allow for more robust weed control options. As per the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), the petitioner

will submit to the Environmental Protection Agency (EPA) metabolism and residue data, along with proposed labeling changes, for the use of 2,4-D on DAS-40278-9 corn (DAS 2010, pages 18 and 113). Under FIFRA, without an experimental use permit issued by the EPA, it is unlawful to use an herbicide “in a manner inconsistent with its labeling” (7 U.S.C. 136j).

The “fop” herbicides (AAOP ACCase inhibitors) have been registered for crop use for over 20 years. They have not traditionally been used to control weed species in corn fields because, as a grass (Poaceae family) species, corn is damaged by AOPP ACCase inhibitor activity. The use of “fop” herbicides has been primarily on broadleaf crops, such as soybean, to control grass weed species, although certain cereal plant varieties have a level of tolerance to some “fops.” According to the USDA National Agricultural Statistics Service (NASS) Agricultural Chemical Use Database, “fop” type herbicides were used for weed control on at least 23 food crop species between 1990 and 2006, totaling over 16 million pounds of active ingredient (<http://www.pestmanagement.info/nass/index.html>, Accessed 3/2010). If a determination of nonregulated status is reached, DAS-40278-9 corn would be the first GE corn variety with nonregulated status that also has resistance to the “fop” class of herbicides. The petitioner has indicated that “fop” herbicides could be used to maintain seed purity in DAS-40278-9 corn breeding nurseries, hybrid production fields, and generally for the control of grass weeds in corn. As required under FIFRA, metabolism and residue data, along with proposed labeling changes will be submitted to the EPA, for the use of “fop”-type herbicides (specifically quizalofop) in DAS-40278-9 corn fields (DAS 2010, page 18). Under FIFRA, it is unlawful to use an herbicide “in a manner inconsistent with its labeling” without an experimental use permit issued (7 U.S.C. 136j). Quizalofop is currently under registration review ([http://www.epa.gov/oppsrrd1/registration\\_review/](http://www.epa.gov/oppsrrd1/registration_review/)) by the EPA with a Final Decision expected sometime in 2013 (EPA-HQ-OPP-2007-1089 at <http://www.regulations.gov>, accessed 3/2010).

Relatively few weed species have developed resistance to synthetic auxins or ACCase inhibitor herbicides compared to several other classes of herbicides. The International Survey of Herbicide Resistant Weeds (ISHRW) regularly updates a website with lists of “field selected, genetically inherited resistant weed biotypes that survive a rate of herbicide to which the indigenous population was controlled” that are discovered and verified globally (<http://www.weedscience.org>, accessed 04/2010). According to the ISHRW, there are 28 broadleaf weeds (8 in the U.S.) that have been selected for resistance to synthetic auxins (including phenoxy auxins such as 2,4-D), and 38 grass weeds (15 in the U.S.) with resistance to ACCase inhibitors (including “fops”). Within the U.S., none of the broadleaf weeds resistant to synthetic auxins have been found in corn fields to date, and grass weeds resistant to “fops” have also not been a problem for corn growers as ACCase inhibitors have not traditionally been used to control weeds in corn fields after planting. By comparison, there are 108 weeds found worldwide that have developed resistance to ALS inhibitor-type herbicides, 68 that have developed resistance to Photosystem II inhibitors (such as atrazine), 18 that have been found to be resistant to glyphosate, and, according to the ISHRW, many of these resistant weeds were found in U.S. corn fields. The petitioner has also indicated that the herbicide resistance traits of DAS-40278-9 corn might be combined (or “stacked”) with other herbicide resistance traits (i.e. glyphosate and glufosinate) (DAS 2010, pages 18, 115, and 151) to use as part of a weed control strategy for growers. Within the U.S. there have not been any weeds that have shown resistance to either glyphosate or atrazine and either a synthetic auxin or a “fop” herbicide. Additionally, there are

no weeds identified within the U.S. that are resistant to both 2,4-D and another class of herbicide, though instances have been identified in other countries. The ISHRW has indicated however, that there are five instances of weeds within the U.S. showing resistance to both ACCase inhibitors and another herbicide mode of action class, though none of these were located within corn fields. The use of DAS-40278-9 corn, regardless of the stacking with other herbicide resistance traits that may or may not occur, will provide growers with another weed control option, and should enable the continued use of no- or reduced-tillage in corn crops in areas where glyphosate herbicide resistant weeds have appeared.

## **Description of Inserted Genetic Material**

DAS-40278-9 corn was produced by transformation of embryogenic suspension cultures of the recipient corn line Hi-II using silicon carbide whisker fibers (Petolino et al. 2003; Petolino and Arnold 2009) to introduce a 6236 base pair restriction fragment expression cassette containing the genetic material that enables the production of the AAD-1 enzyme (DAS 2010, page 21). The petitioner has established that DAS-40278-9 corn contains only this expression cassette as described in the petition (DAS 2010, pages 26-57) and that this inserted genetic material results in the production of the AAD-1 protein within the DAS-40278-9 corn plants (DAS 2010, pages 58-63).

Corn plants containing the inserted genetic material were initially isolated by growing transformed plant tissue on medium containing *R*-haloxyfop, an AOPP ACCase inhibitor (DAS 2010, page 21). Whole plants were generated from the surviving tissue and selected plants were bred with desirable corn lines to generate elite corn varieties containing the genetic insertion. DAS confirmed via Southern blot analyses that the final corn product (DAS-40278-9) does not contain any plasmid sequences outside of the intended 6236 base pairs and contains only a single intact insert in the corn genome with no evidence of duplicated or missing DNA from that insertion. Inheritance patterns of the inserted genetic material also demonstrate the intactness and functional stability of the inserted DNA over several breeding generations. (DAS 2010, pages 30-57).

A detailed description of the inserted genetic material is provided in the petition (DAS 2010, pages 26-27), and is summarized below:

The introduced gene responsible for conferring the herbicide resistance encodes an aryloxyalkanoate dioxygenase (AAD-1) enzyme. The gene, *aad-1*, was originally obtained from the soil bacterium *Sphingobium herbicidovorans*, and was subsequently altered to be plant-optimized (the original nucleic acid sequence was changed because plants favor different codons for optimal DNA-protein translation). The final protein product (AAD-1) produced in DAS-40278-9 corn is nearly identical (99.3% identity) to the protein produced by *S. herbicidovorans* differing only by the addition of one amino acid, alanine, at position 2 (DAS, page 58; Wright et al. 2009). DAS provided evidence that this single amino acid change did not impact the function of the enzyme nor was there any evidence that the protein contributed to any changes to the agronomic properties, disease susceptibility, or composition of DAS-40278-9 corn compared to other commercially available corn (see “Potential Impacts of Genetic Modifications on Altered Disease and Pest Susceptibilities” below).

The *aad-1* gene is flanked on either side by regulatory (non-coding) sequences that have been derived from the corn genome. In front of *aad-1* (on the 5' end) is the *Z. mays* ubiquitin 1 (ZmUbi1) promoter which is a sequence of DNA that promotes the constitutive expression of genetic material, particularly in monocots (Christensen et al. 1992, Christiansen and Quail 1996). This particular promoter sequence was previously used in a plant product that has been previously determined to have nonregulated status (APHIS BRS Petition 03-181-01p) and the use of that sequence in DAS-40278-9 corn does not raise any new issues. On the downstream side of the *aad-1* gene is the *Z. mays* peroxidase 3' untranslated region (ZmPer5 3' UTR), which is a regulatory sequence of corn DNA that promotes the efficient transcription and translation of a corn gene (Ainley et al. 2002). Both the ZmUbi1 promoter and the ZmPer5 3' UTR genetic sequences serve only as a regulatory role, and are not translated with the AAD-1 protein (DAS 2010, Appendix 2, pages 129-131).

On either side of the above genetic material, DAS has also included the RB7 Matrix attachment region (MAR). This genetic material, derived from tobacco (*Nicotiana tabacum*) has been demonstrated to improve the stability of expression of inserted genetic material by binding to nuclear matrices within a plant cell (Hall et al. 1991; Allen et al. 2000; Mankin et al. 2003). MAR sequences do not encode for a protein product and are also considered solely as regulatory elements. Also part of the 6236 base pair introduced fragment are short intervening DNA sequences that were part of the original cloning plasmid. These sequences do not contain any coding sequence but represent the linking portions of the plasmid that lie between the DNA sequence insertion points for the regulatory regions and coding region of the expression cassette.

In summary, the only portion of the introduced DNA fragment that is translated into a protein product or that could potentially alter the nutritional or fundamental component characteristics of the corn plants is the *aad-1* gene. An analysis of the impacts of that insertion within DAS-40278-9 corn as it pertains to plant pest risk is presented through the rest of this document.

## **Plant Pest Risk Assessment**

APHIS is responsible for regulating GE organisms and plants under the plant pest provisions in the Plant Protection Act (PPA) of 2000, as amended (7 USC § 7701 *et seq.*) to ensure that they do not pose a plant pest risk to the environment. APHIS regulations at 7 Code of Federal Regulations (CFR) Part 340, which were promulgated pursuant to authority granted by the PPA, 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, vector, or vector agent used in engineering the organism belongs to one of the taxa listed in the regulation (7 CFR 340.2) and is also considered a plant pest. A GE organism is also regulated under Part 340 when APHIS has reason to believe that the GE organism may be a plant pest or APHIS does not have information to determine if the GE organism is unlikely to pose a plant pest risk.

A person may petition the agency that a particular regulated article is unlikely to pose a plant

pest risk, and therefore, is no longer regulated under the plant pest provisions of the PPA or the regulations of Part 340. The petitioner is required to provide information under §340.6(c)(4) to demonstrate that the regulated article that is the subject of the petition does not pose a plant pest risk and that it should no longer be subject to the regulations of 7 CFR part 340. A GE organism is no longer subject to the regulatory requirements of Part 340 or the plant pest provisions of the PPA when APHIS determines that it is unlikely to pose a plant pest risk.

As described earlier in this document, the introduced genetic material in DAS-40278-9 corn confers enhanced tolerance and resistance to treatment with select herbicides to the GE plants. No plant pest or plant pest-derived material was used to generate the DAS-40278-9 corn plants. DAS has planted DAS-40278-9 corn as a regulated article under APHIS BRS Notification of Environmental Release since 2006 (DAS 2010, page 144).

Potential impacts to be addressed in this plant pest risk assessment are those that pertain to the use of DAS-40278-9 corn and its progeny in the absence of confinement as otherwise afforded under conditions of 7 CFR part 340 for the introduction of genetically engineered organisms. Of the information requested by APHIS for submission of a petition for nonregulated status, APHIS examined information submitted by the petitioner (as per 7 CFR part 340.6(c)(4)) related to plant pest risk characteristics, disease and pest susceptibilities, expression of the gene product, new enzymes or changes to plant metabolism, weediness (including control options) of the regulated article, the possibility of effects of the regulated article on non-target organisms, and any impacts on the weediness of any other plant with which it can interbreed. Other issues related to agricultural or cultivation practices (including but not limited to those related to changes in herbicides used to control weeds in corn), non-target organisms, beneficial organisms, threatened and endangered species and other environmental impacts have been addressed in an Environmental Assessment (EA) that has been prepared to analyze potential impacts of a determination of nonregulated status of DAS-40278-9 corn by APHIS.

## **Potential Impacts of Genetic Modifications on Altered Disease and Pest Susceptibilities**

APHIS assessed whether DAS-40278-9 corn is likely to have significantly increased disease and pest susceptibility compared to conventional corn. This assessment encompasses a thorough consideration of introduced traits and interactions with pests and disease.

Corn (*Zea mays*) is not a plant pest in the United States (USDA-APHIS 2000). Furthermore, none of the inserted genetic sequences are derived from any plant pests listed in 7 CFR part 340.2 or pests listed elsewhere in 7 CFR parts 300-399. The description of the genetic modification, including the introduced genetic elements and the resulting expression and function of the AAD-1 protein product in DAS-40278-9 corn, has been summarized above. DAS routinely monitored their corn field trials for corn diseases such as ear rots, leaf blights, rusts, and leaf spots along with insect damage from corn pests such as leaf hoppers, mites, thrips, aphids, beetles, grasshoppers, and corn earworms. DAS conducted agronomic field trials during the 2008 growing season across 27 locations representative of the major corn-growing areas of the U.S. (DAS 2010, pages 64-75). There were no statistically significant differences in agronomic characteristics such as vigor, lodging or yield that might be indicative of altered

sensitivity to pests and diseases between DAS-40278-9 corn, derived hybrids and their control counterparts under standard corn management practices (DAS 2010, page 70) or in response to specific herbicide treatments (DAS 2010, page. 67). In one experiment, disease and insect damage ratings were taken for treatments that included DAS-40278-9 hybrid corn sprayed with 2,4-D, quizalofop, 2,4-D and quizalofop, and neither herbicide, as well as the near-isoline control hybrid planted at six (6) field sites; Richland, IA; Carlyle, IL; Wyoming, IL; Rockville, IN; York, NE; and Branchton, Ontario, Canada (DAS 2010, page 71). The data submitted by DAS confirmed that the inserted genetic material did not confer any altered disease or pest susceptibility to DAS-40278-9 corn. Specific disease and insect stressor observations were collected from 2006 to 2008 across locations representing a broad range of U.S. corn growing regions (DAS 2010, pages 64-75).

The data presented in the petition (DAS 2010, pages 76-101) indicate no material difference in compositional and nutritional quality of DAS-40278-9 corn compared to other commercially available corn apart from the presence of the AAD-1 protein. A few variables did show statistically significant differences between the compositional characteristics of DAS-40278-9 corn and control corn grown at the same locations. However, these differences were relatively small and none of the values for the forage and grain composition characteristics were outside the range of natural variability of conventional corn reported by the International Life Sciences Institute Crop Composition Database (Ridley et al. 2004; ILSI 2006) or in the OECD consensus document on corn composition (OECD 2002). Therefore, with the exception of the AAD-1 protein which is at most 0.02% of corn tissue dry weight (range: 0.42-210 ng/mg depending on the tissue, see DAS 2010, page 20), the composition of DAS-40278-9 corn is not biologically different than conventional corn. Data in the petition (DAS 2010, pages 68-70) showed no significant difference in grain yield between a DAS-40278-9 corn hybrid and a near isogenic control line across 21 locations (6 states). These data collectively support the petitioner's claim that DAS-40278-9 corn does not have increased susceptibility to any insects, plant pathogens, or other plant pests over its non-transgenic control.

The AAD-1 protein is derived from a gram-negative soil bacterium *Sphingobium herbicidovorans* that is not associated with plant disease (Balkwill et al. 2006; Kohler 1999; Zipper et al. 1996; Horvath et al. 1990). *S. herbicidovorans* was originally isolated using a soil-column enrichment followed by culture growth with phenoxy auxin herbicide as the sole carbon and energy sources (Horvath et al. 1990). The AAD-1 protein expressed by the DAS-40278-9 corn, with the exception of the second amino acid is identical to proteins found in other soil bacteria (Non-redundant Protein Basic Local Alignment Search Tool (BLAST), <http://www.ncbi.nlm.nih.gov/>, accessed 4/2010), including *Rhodospirillum rubrum* P230 and *Delftia acidovorans*, which have also been isolated from herbicide-enriched substrate (Müller et al. 2001) and are not considered plant pests (Wen et al. 1999). Using BLAST, the AAD-1 protein was classified as an R-2,4-dichlorophenoxypropionate dioxygenase, and it has also been named as an (R)-dichloroprop/α-ketoglutarate dioxygenase (RdpA) (Müller, et al. 2004; Wright et al. 2009). The enzyme has only been associated with the metabolism of herbicides and there is no indication that it has any other function.

In summary, all evidence reviewed by APHIS indicate that DAS-40278-9 corn is just as susceptible to plant pathogens and other plant pest species as conventional corn and that there is no added plant pest risk that results from the insertion and expression of the genetic material



described in the petition.

## **Potential Impacts Based on the Relative Weediness of DAS-40278-9 Corn**

Corn is not typically considered as a weed (Crockett 1977; Holm et al. 1979; Muenscher 1980) and is not listed on the Federal noxious weed list (7 CFR part 360). Corn possesses few of the characteristics of notably successful weeds and corn is grown as a crop throughout the world without any report that it is a serious weed or that it forms persistent feral populations (Baker 1965; Keeler 1989). However, corn seed can germinate in undesired locations and would then be considered a weed. A common example of this is the appearance of corn seedlings in soybean fields following a corn crop which has been associated with reduced soybean yields (Beckett and Stoller 1988). Compared to other corn varieties, DAS-40278-9 has improved fitness (reduced injury) in the presence of certain herbicides, which translates into fewer options for the removal of volunteer plants. Nonetheless, there are many available options for the control of DAS-40278-9 plants so that growers can avoid losses in fields where unwanted DAS-40278-9 might be growing.

APHIS assessed whether DAS-40278-9 corn is any more likely to become a weed than any other corn varieties currently under cultivation. This assessment encompasses a thorough consideration of the basic biology of corn and an evaluation of the unique characteristics of DAS-40278-9 corn evaluated under field conditions. DAS conducted agronomic field trials during the 2008 growing season across 27 locations representative of the major corn-growing areas of the U.S. (DAS 2010, pages 64-75). There were few statistically significant differences between DAS-40278-9 corn, derived hybrids and their control counterparts, regardless of herbicide treatment. The significant differences that were seen (DAS 2010, page 67), in ear height and a visual estimate of plant health (stay green), reflected very small differences in morphological features and were not accompanied by significant overall treatment effects (DAS 2010, page 66). No differences in phenotypic characteristics that would contribute to enhanced weediness were observed between DAS-40278-9 corn and control lines for the range of phenotypic endpoints assessed.

Based on the agronomic field data and literature survey about corn weediness potential, DAS-40278-9 corn lacks the ability to persist as a troublesome or invasive weed. Still, because DAS-40278-9 corn might grow in areas where it is undesired, the GE corn, similar to other corn varieties, would be considered a weed (Beckett and Stoller 1988). Because the genetic elements inserted into DAS-40278-9 corn confers greater tolerance or resistance to herbicides the measures that can be used to remove volunteer DAS-40278-9 corn are more limited compared to other corn varieties. Nonetheless, as discussed in the next paragraphs, most of the currently used control measures for removing volunteer corn are also available for the removal of DAS-40278-9 corn.

Corn is commonly grown in rotation with other crop varieties including, but not necessarily limited to, oats, forage crops, peanut, wheat, rye, cotton, and soybean. Corn seed left in a field after harvest is common, and that seed can germinate, or “volunteer,” during the growth of the following rotational crop. If this corn is not destroyed or removed, the subsequent crop planting may have lower yields and reduced value due to competition and mixing with the volunteer corn

(Beckie and Owen 2007; Beckett and Stoller 1988). The advent and wide adoption of herbicide resistant crops has already changed the approaches growers can adopt to reduce crop yield losses resulting from volunteer corn. For example, glyphosate-resistant soybean plants, grown after glyphosate-resistant corn, can have reductions in soybean yield, due to competition with volunteer corn, if glyphosate is the only weed control method used (Soltani et al. 2006). Similar studies have also been done for corn-cotton rotations (Clewis et al. 2008; Thomas et al. 2007) and corn-wheat rotations (Martin 2008).

One suggested chemical method for the control of volunteer corn among broadleaf crops is the use of graminicide ACCase inhibitors which include “fops” (Deen et al. 2006; Soltani et al. 2006). However, the use of “fop” ACCase inhibitor herbicides would not be effective for the control of volunteer DAS-40278-9 corn due to the resistance that has been conferred by genetic engineering. Nonetheless, other ACCase inhibitor graminicides, such as cyclohexanedione (“dim”) herbicides (e.g. clethodim or sethoxydim) are also effective for the control of volunteer corn in broadleaf rotational crops (Clewis et al. 2008; Thomas et al. 2007; Deen et al. 2006; Soltani et al. 2006; WSSA 2007), although the level of control with sethoxydim has been reported to be less effective at manufacturer recommended doses than the “fop” herbicides tested (Soltani et al. 2006). Another large class of herbicides that can be used for the elimination of volunteer corn includes the acetolactate synthesis (ALS) inhibitors, such as imazamox, imazaquin, and imazethapyr (WSSA 2007). The petitioner has confirmed the susceptibility of DAS-40278-9 corn to both “dim” and ALS inhibitor herbicides at labeled-use levels (DAS 2010, page 115 and April 29, 2010 correspondence with APHIS). ALS inhibitor herbicides as well as paraquat can also be used to control volunteer corn in corn to wheat rotations (Martin 2008).

Other commonly used methods for the control of volunteer corn are also still available for use on DAS-40278-9. For example, non-selective herbicides (e.g. glyphosate and glufosinate) are options for eliminating DAS-40278-9 corn and its progeny, assuming that there has been no breeding (or stacking) or cross-pollination with other corn lines that have resistance traits to those herbicides. Use of any herbicide for the control of volunteer DAS-40278-9 corn is subject to any labeling restrictions established by the EPA, as per FIFRA. Additionally, there are also many non-chemical methods of weed control (tilling, mechanical removal, etc.) that are effective for removing any unwanted DAS-40278-9 corn.

The possibility of herbicide-resistant volunteer corn competing with other crops is not confined to GE varieties. Other cultivar-development methods have generated corn varieties that have reduced sensitivity to herbicides that could otherwise control for volunteer corn in crops such as soybean. For example, there are commercially available corn hybrid varieties with resistance to sethoxydim, a cyclohexanedione ACCase inhibitor (Vangessel et al. 1997). Similar to DAS-40278-9, many options for control of the herbicide resistant corn are available (Young and Hart, 1997). While farmers may have to change their management strategies due to DAS-40278-9, these changes will not necessitate a major departure from well-established and broadly used agricultural protocols.

## **Potential Impacts from Outcrossing (Gene Flow) to Sexually-compatible Wild Relatives**

Gene flow is a natural biological process with significant evolutionary importance. A number of angiosperm taxa are believed to be derived from hybridization or introgression between closely related taxa (Grant 1981; Soltis and Soltis 1993; Rieseberg 1997; Hegde et al. 2006). Even in existing floras, the occurrence of hybridization or introgression is reported to be widespread (Knobloch 1972; Stace 1987; Rieseberg and Wendel 1993; Peterson 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 other crops (Ellstrand et al. 1999).

APHIS evaluated the potential for gene introgression to occur from DAS-40278-9 corn to sexually compatible wild relatives and considered whether such introgression would result in increased weediness. Cultivated corn, or maize, *Zea mays* L. subsp. *mays*, is sexually compatible with other members of the genus *Zea*, and to a much lesser degree with members of the genus *Tripsacum* (OECD 2003). Wild diploid and tetraploid members of *Zea*, collectively referred to as teosinte, are normally confined to the tropical and subtropical regions of Mexico, Guatemala, and Nicaragua (Wilkes 1967; Fukunaga et al. 2005). In the U.S. isolated populations of teosinte have been reported in Florida (<http://www.florida.plantatlas.usf.edu/>, Taba 1995).

The genus *Tripsacum* contains up to 16 recognized species, most of which are native to Mexico, Central and South America, but three (*T. dactyloides*, *T. floridatum*, and *T. lanceolatum*) exist as wild and/or cultivated species in the continental U.S. (OECD 2003); and two taxa (*T. fasciculatum* and *T. latifolium*) also occur in Puerto Rico (USDA, NRC, 2010). Though many of these species occur where corn might be cultivated, gene introgression from DAS-40278-9 corn under natural conditions is highly unlikely. Hybrids of *Tripsacum* species with *Zea* are difficult to obtain outside of a laboratory and are often sterile or have greatly reduced fertility, and none of them can withstand even the mildest winters. Furthermore, 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).

Introgression of genes from corn into teosinte or *Tripsacum* species in the U.S. has not been described to occur in nature. While some teosinte may be considered weeds in certain instances, they are also used by some farmers for breeding improved corn (Sánchez and Ruiz, 1997, and references therein). Teosinte is described as being susceptible to many of the same pests and diseases that attack cultivated corn. In the wild, introgressive hybridization from corn to teosinte is currently limited by several factors including geographic isolation, differing degrees of genetic incompatibility, differences in flowering time in some cases, developmental morphology and timing of the reproductive structures, dissemination, and dormancy (Doebley 1990a and 1990b; Galinat 1988; Ellstrand 2007). First-generation hybrids are generally less fit for survival and dissemination in the wild, and show substantially reduced reproductive capacity, which serves as a significant constraint to introgression. Guadagnuolo et al. (2006) did report that hybrids between a glyphosate-resistant maize cultivar and “chalco teosinte” (*Z. mays* ssp. *mexicana*) showed greater vigor and produced more seeds than the wild parent. Nonetheless, in the absence of selective pressure from glyphosate there was no direct positive or negative impact of the transgene on the fitness or vigor of either the hybrids or the pure maize progeny.

Data included in the petition demonstrated that there were no significant differences in viability and morphology of pollen collected from DAS-40278-9 corn and control corn (DAS 2010, pages

73-74) and therefore the out-crossing rate of DAS-40278-9 corn is not expected to be different compared to other corn varieties. Based on the data presented in the petition, DAS-40278-9 corn does not exhibit characteristics that cause it to be any weedier than other cultivated corn and the extremely limited potential for gene introgression into teosinte or *Tripsacum* species is not expected to be any different than that of other cultivated corn varieties. Even in the unlikely event that gene flow were to occur between DAS-40278-9 corn and wild relatives, there is no reason to expect that the possible expression of *aad-1* would transform corn wild relatives into more weedy species, especially in the absence of herbicide selection pressure. Hybrids, if they occurred, could possibly have diminished susceptibility to some herbicides but would still be susceptible to many others, as is DAS 40278-9.

In summary, DAS-40278-9 corn will not adversely impact sexually compatible wild relatives or the weediness attributes of those wild relatives.

### **Potential Impacts of DAS-40278-9 on Organisms Considered Beneficial to Agriculture**

The petitioner also assessed the potential for DAS-40278-9 corn to impact “non-target” organisms, including those considered beneficial to agriculture, and determined that there would be no effect (DAS 2010, page 116). DAS-40278-9 is agronomically and compositionally similar to other corn varieties and will not adversely affect other organisms compared to other corn varieties. Furthermore, APHIS has found no evidence or reason to believe that the presence of the *aad-1* gene or the expressed AAD-1 protein in DAS-40278-9 corn would have any impact on other organisms, including organisms beneficial to agriculture (such as earthworms, honeybees, predators or parasites of corn pests). The AAD-1 protein is expressed in a variety of plant tissues in DAS-40278-9 corn with average expression values ranging from 2.87 ng/mg dry weight in R1 stage root to 127 ng/mg in pollen tissue (DAS 2010, page 60) and expression values were similar for sprayed treatments as well as for plots sprayed and unsprayed with 2,4-D and quizalofop herbicides. The inserted genetic material is not secreted, is not toxic, and does not produce any substance that is secreted or that would be considered toxic. The AAD-1 protein does not share meaningful amino acid sequence similarities with known toxins based on information reported by DAS (DAS 2010, page 62). Amino acid homologies were evaluated using a global sequence similarity search against the GenBank non-redundant protein dataset and the only significant homologies identified were with other alpha-ketoglutarate dependent dioxygenases, the same class of enzymes as AAD-1. DAS reported that none of the similar proteins returned by the search identified any safety concerns that might arise from the expression of AAD-1 protein in plants. The *aad-1* gene, isolated from a soil bacterium, and the AAD-1 protein are both present in nature. The soil bacterium, *Sphingobium herbicidovorans*, is neither a plant pest nor a known pest of organisms beneficial to agriculture and the AAD-1 protein is similar to proteins found in other soil bacteria that are also not considered pests (Wright et al. 2009; Balkwill et al. 2006).

Any effects on non-target organisms that could potentially result from proposed changes in herbicide labels will be evaluated by the EPA.

### **Potential to Transfer Genetic Information from DAS-40278-9 Corn to Organisms with which it cannot Interbreed**

APHIS examined the potential for the genetic material inserted into DAS-40278-9 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, including the creation of more virulent plant pests. The horizontal gene transfer (HGT) between unrelated organisms is one of the most intensively studied fields in the bio-sciences since 1940, and the issue gained extra attention with the release of transgenic plants into the environment (Dröge et al. 1998). Potential risks from stable HGT from genetically engineered organisms to another organism without reproduction or human intervention was recently reviewed (Keese 2008). Mechanisms of HGT include conjugation, transformation and transduction, and other diverse mechanisms of DNA and RNA uptake, recombination, and rearrangement, that occur most notably through viruses and mobile genetic elements. HGT has been implicated as a contributor to major transitions in evolution, including the spread of antibiotic resistance amongst pathogenic bacteria and the emergence of increased virulence in bacteria, eukaryotes, and viruses.

### **Potential for Horizontal Gene Transfer to Bacteria or Fungi**

HGT 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 and bacteria (e.g. as commensals, symbionts, parasites, pathogens, decomposers, or in the guts of herbivores), there are almost no evolutionary examples of HGT to bacteria from eukaryotes or from plants to fungi (as reviewed in Keese 2008). The only genes likely to be transferred successfully from genetically engineered plants to bacteria are other bacterial genes. Horizontal transfer of the *aad-1* gene construct from the nuclear genome of DAS-40278-9 corn and subsequent expression in bacteria is unlikely to occur. First, many genomes (or parts thereof) have been sequenced from bacteria that are closely associated with plants including *Agrobacterium* and *Rhizobium* (Kaneko et al. 2000; Wood et al. 2001; Kaneko et al. 2002). There is no evidence that these organisms contain genes derived from plants. Second, in cases where review of sequence data implied that HGT occurred, these events are inferred to occur on an evolutionary time scale on the order of millions of years (Koonin et al. 2001; Brown 2003). Third, the 6236 base pair insertion made to confer the herbicide tolerance to DAS-40278-9 corn is a small fraction of a percent (approximately 0.00025%) of the total DNA (approximately 2500 million base pairs) found in the corn genome. Forth, transgene DNA promoters and coding sequences are optimized for plant expression, not prokaryotic bacterial expression so even if HGT occurred, proteins corresponding to the transgenes are not likely to be produced. Finally, the FDA evaluated HGT 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).

### **Potential for Horizontal Gene Transfer to Viruses**

APHIS also considered whether horizontal transfer of DNA from DAS-40278-9 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 (for a general review of the

issue see Keese 2008). Because there are no virus sequences used as part of the inserted gene cassette and no sequences that have been implicated in viral recombination or pathogenicity, there is no reason to think that viral recombination of the herbicide-resistance conferring genetic material will occur, or that such theoretical recombination would have any impact on plant pest risk.

### **Potential for Horizontal Gene Transfer to Parasitic Plants**

Recently, Yoshida et al. (2010) through a comparative genomics analysis implicated HGT for the incorporation of a specific genetic sequence in the parasitic plant purple witchweed (*Striga hermonthica*), which infests cereal fields (monocots) including corn and sorghum (*Sorghum bicolor*). According to this study, the incorporation of the specific genetic sequence (with an unknown function) 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 between a parasitic plant and its host is an extremely rare event, and furthermore, *S. hermonthica* is not found in the U.S. and *S. asiatica*, another related parasite of cereal crops, is only present in North Carolina and South Carolina (USDA, NRC, 2010).

For all the reasons listed above, APHIS concludes that HGT is unlikely to occur and thus poses no plant pest risk.

### **Conclusion**

APHIS has reviewed and conducted a plant pest risk assessment on DAS-40278-9 corn. Due to the lack of plant pest risk from the inserted genetic material, the lack of weediness characteristics of DAS-40278-9 corn, the lack of atypical responses of DAS-40278-9 corn 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 concerns of horizontal gene transfer, APHIS concludes that DAS-40278-9 corn is unlikely to pose a plant pest risk.

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