Bayer CropScience LP Request (16-235-01p) for the Extension of Determination of Non-regulated Status for Male Sterile, Glufosinate Ammonium Tolerant *Brassica napus* (Canola) Event MS11

OECD Unique Identifier: BN012-7

Plant Pest Risk Similarity Assessment

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A. Introduction

The Animal and Plant Health Inspection Service (APHIS) of the United States Department Agriculture (USDA) has received an extension request (petition number 16-235-01p) from Bayer CropScience LP (hereafter referred to as Bayer). In accordance with §340.6(e)(2), Bayer requests that APHIS extend the non-regulated status for antecedent canola event MS8 with male sterility and glufosinate tolerance to the genetically engineered (GE) MS11 event and any progeny derived from crosses of the MS11 event with conventional canola, and any progeny derived from crosses of the MS11 event with other GE canola varieties that have received a determination of non-regulated status, or are not considered regulated articles under regulations at 7 CFR Part 340.

Earlier USDA announced its determination of non-regulated status for two *Brassica napus* (canola) events and their hybrid progeny (petition number 98-278-01p, AgrEvo USA Company, 1998) on March 22, 1999. These two events were:

- MS8 - Drakkar variety of *Brassica napus* (canola) for male sterility and glufosinate tolerance;
- RF3 – Drakkar variety of *Brassica napus* (canola) for restoration of male sterility and glufosinate tolerance.

Presently, Bayer intends to pursue commercialization of the MS11 event that confers both male sterility and glufosinate tolerance in the parent line N90-740. The antecedent organism identified in the extension request for the MS11 Event is event MS8 (hereafter referred to as the Bayer antecedent MS8 event[1]).

APHIS regulations in 7 CFR part 340 regulate the introduction (importation, interstate movement, or release into the environment) of certain GE organisms and products. A GE organism is no longer subject to the regulatory requirements of Part 340 when APHIS determines that it is unlikely to pose a plant pest risk. A GE organism is considered a regulated article under Part 340 if the donor organism, recipient organism, or vector, or vector agent used in engineering the organism belongs to any genera or taxa designated in 7 CFR 340.2 and meets the definition of plant pest, or is an unclassified organism and/or an organism whose classification is unknown, or any product which contains such an organism, or any other organism or product altered or produced through genetic engineering which the Administrator determines is a plant pest or has reason to believe is a plant pest. The MS11 event was produced by the *Agrobacterium tumefaciens*-mediated transformation of canola (Bayer CropScience, 2016), and some of the introduced border sequences come from plant pest organisms listed in 7 CFR 340.2 (Bayer CropScience, 2016). Therefore, the MS11 event is considered a regulated article under APHIS regulations at 7 CFR part 340.

[1] AgrEvo USA Company, which generated the MS8 event, became Aventis CropScience in 1999 following a merge with Rhône-Poulenc. The resulting company, Aventis CropScience, was then acquired by Bayer CropScience in 2001.

Potential impacts in this Plant Pest Risk Similarity Assessment are those that pertain to plant pest risk associated with the MS11 event and its progeny and their use in the absence of confinement relative to the Bayer antecedent canola event, MS8. APHIS utilizes data and information submitted by the applicant, in addition to current literature, to determine if the MS11 event is any more likely than the Bayer antecedent canola event MS8 to pose a plant pest risk. APHIS specifies in 7 CFR 340.6(e) that an extension request for non-regulated status shall include information to establish the similarity of the antecedent organism to the regulated article in question.

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

B. Development of the MS11 Canola

In 1998, Bayer genetically engineered two traits of interest to canola producers and processors into a commercial canola variety (Drakkar) with low-erucic acid in its oil and low glucosinolate content in its meal by-product (both anti-nutrients). These traits were male sterility and glufosinate tolerance (Event MS8; petition number 98-278-01p; AgrEvo USA Company, 1998). With Event MS11, these traits have now been transformed into a new canola variety (N90-740) that also expresses low anti-nutrient levels of erucic acid and glucosinolate. MS11 additionally contains a male sterility restorer gene (used in event RF3, which was granted non-regulated status in 1999; USDA-APHIS-BRS, 1999). The same gene cassettes used for antecedent event MS8, and a similar gene cassette used for event RF3, were used to generate canola event MS11. The intended purpose of the Bayer MS11 event is to provide the canola producers and processing industry with a new canola line with male sterility and glufosinate tolerance. The low level of the male fertility restoration gene expressed in the MS11 event facilitated transformation of N90-740 canola by *Agrobacterium tumefaciens* and does not affect male sterility of the MS11 event in canola as a whole.

APHIS BRS completed a detailed plant pest risk assessment (PPRA) and environmental assessment (EA) for the Bayer antecedent canola event MS8 (USDA-APHIS-BRS, 1999). The EA addressed all resource areas of potential concern. In the antecedent petition, 98-278-01p, APHIS concluded on the basis of the EA that the impacts would not be significant. The agency issued a Finding of No Significant Impact (FONSI) and made a determination of non-regulated status for Events MS8 and RF3 on March 22, 1999.

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

To inform APHIS of the potential hazards resulting from the genetic modification and potential routes of exposure related to the inserted DNA and its expression products, APHIS assessed data and information presented in the extension request related to the similarity of the MS11 event to the Bayer antecedent canola event MS8, including information about: the transformation process; the source of the inserted genetic material and its function in both the donor organism and the GE crop event; the integrity, stability and mode of inheritance of the inserted genetic material through sexual or asexual reproduction; and the number of loci inserted.

APHIS also assessed data presented in the extension request on whether the genetic modification results in expression of new genes, proteins, or enzymes, suppression of existing genes and their products. APHIS reviewed the data from the antecedent MS8 event and determined the antecedent had no major changes in oil or seed protein content and composition; therefore, it is not anticipated that the MS11 event will cause changes to metabolites or compounds which could affect plant pest risk. The assessment encompasses a consideration of any observed or anticipated effects on plant metabolism including, e.g. any relevant changes in levels of metabolites, anti-nutrients, or nutrients in harvested canola derived from the MS11 event compared to the Bayer antecedent canola event or those in the conventional counterparts and other comparators.

Description of the genetic modification and inheritance of inserted DNA

Transformation of the MS11 event (*Brassica napus*) was accomplished through *Agrobacterium tumefaciens*-mediated transformation of canola from the N90-740 parental line as described in Bayer's extension request (Bayer CropScience, 2016, p. 15). This parental line has the same low anti-nutrient attributes as the Drakkar parental variety used in the antecedent canola event MS8 (Bayer CropScience, 2016, p 11).

The same gene cassette (Bayer CropScience, 2016, p. 21) used to transform the Bayer antecedent canola event MS8, was used to transform and generate the MS11 canola event. The T-DNA genetic construct for event MS11 contains DNA sequences intended to confer male sterility and glufosinate tolerance. The MS11 event also contains DNA sequences from another non-regulated GE canola that confers low levels of a male sterility restorer protein (event RF3) that facilitates *Agrobacterium tumefaciens*-mediated transformation (AgrEvo USA Company, 1998).

- The antecedent canola line event MS8, has two coding genes; one that causes male sterility and one that confers glufosinate resistance.
    - The male sterility is conferred by the insertion of the coding region of the *barnase* gene. This gene is driven by the promoter region of the anther-specific gene, *TA29*. This promoter is comprised of 1.5 kb of the *TA29* sequence upstream from the ATG initiation codon. The anther promoter drives the *barnase* gene specifically at the anther region of the plant

causing cell death and thus, male sterility. There are two terminator sequences for the *barnase* gene: the 3' untranslated region downstream from the *barnase* coding sequence followed by a 260 bp *TaqI* fragment from the 3' untranslated end of the nopaline synthase gene (*3'nos*) containing plant polyadenylation signals.

- The glufosinate resistance is derived from the coding sequence of the bialaphos resistance gene (*bar*). This gene is driven by the promoter region of the atS1A ribulose-1,5-biphosphate carboxylase small subunit gene (*PSsuARA*). The terminator for the *bar* gene consists of the 3'untranslated region from the TL-DNA gene 7 (*3'g7*).

- The prior non-regulated GE canola line event RF3, has two coding genes; one that restores male fertility and one that confers glufosinate resistance.

  - The restoration of male fertility is conferred by the insertion of the coding region of the *barstar* gene. This gene is driven by the promoter region of the anther-specific gene, *TA29*. The promoter drives the *barstar* gene specifically at the anther region of the plant preventing deformation of the anther, and thus, restoring male fertility. There are two terminator sequences for the *barstar* gene: the 3' untranslated region downstream from the *barstar* coding sequence followed by a 260 bp *TaqI* fragment from the 3' untranslated end of the nopaline synthase gene (*3'nos*) containing plant polyadenylation signals.

  - The glufosinate resistance is derived from the coding sequence of the bialaphos resistance gene (*bar*). This gene is driven by the promoter region of the atS1A ribulose-1,5-biphosphate carboxylase small subunit gene (*PSsuARA*). The terminator for the *bar* gene consists of the 3'untranslated region from the TL-DNA gene 7 (*3'g7*).

- The MS11 event contains the exact coding genes and regulatory constructs as the antecedent event, MS8. The MS11 also contains the exact coding sequences found in the previously non-regulated canola event RF3, but the *barstar* gene is regulated by a different and weaker promoter and a different terminator (*pNos* from *Agrobacterium tumefaciens* and *3'g7* from *Agrobacterium tumefaciens*, respectively) resulting in minimal expression of the male fertility restorer gene to facilitate *Agrobacterium*-mediated transformation, but not change the male sterility phenotype of the MS11 event.

APHIS reviewed the information provided by Bayer in the extension request and determined the following:

- The MS11 event contains a single, intact copy of the *barnase* gene, *barstar* gene and *bar* gene with left and right border repeats from the pTCO113 T-DNA.
- The T-DNA is stably inherited from generation to generation.

- The MS11 event does not contain any backbone sequence of extraneous DNA fragments from the transformation plasmid, pTCO113.
- The MS11 event contains no marker genes.
- The MS11 event contains a stable, well-characterized insert.
- During the transformation process for event MS11, 24 base pairs of the left border sequence and 24 base pairs of the right border sequence of the T-DNA were truncated. These sequences are outside of the functional DNA elements and do not impact expression of the transgenes.

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

Sequence analysis of the MS11 *B. napus* insert confirmed the sequence of the *barnase* gene was as expected. Gene product expression of *barnase* in event MS11 was tested on whole plant during leaf development, stem elongation, and inflorescence; root during stem elongation and inflorescence; raceme during inflorescence; and grain at maturity. The levels of gene product expression could not be directly compared to the MS8 event since expression was below the limit of detection; however, the MS11 *B. napus* plants exhibited the same male-sterile phenotype as the antecedent organism MS8, demonstrating the presence of the active barnase protein (AgrEvo USA Company, 1998; Bayer CropScience, 2016).

The *barstar* gene product was produced at lower levels than the previously deregulated event RF3 by using a weak promoter (*pNos*) which did not negate the male-sterile phenotype of event MS11, but was inserted to enhance *Agrobacterium*-mediated transformation. Sequence analysis confirmed the sequence of the *barstar* in the MS11 event was as expected, but the gene product was only consistently detected in the roots. The protein expression was below detectable levels in grain, raceme, and whole plants. The protein testing method used in event MS11 was not comparable to the previously deregulated canola event RF3 (the event containing the *barstar* gene), but the sequencing data and the subsequent enhancement of *Agrobacterium*-mediated transformation for event MS11 indicated the presence of the *barstar* gene and gene product (AgrEvo USA Company, 1998; Bayer CropScience, 2016).

The PAT protein was extracted and tested from the leaves of the event MS11 plants to the test the presence of the *bar* gene product. The testing methods were not comparable to the testing method for the MS8 event, but the agronomic behavior and weight of evidence of the testing of extracted PAT protein from MS11 event demonstrated comparable glufosinate resistance; and therefore, similar results as the antecedent event, MS8 (AgrEvo USA Company, 1998; Bayer CropScience, 2016).

The barnase and barstar proteins have a long history of safe use in canola since granted non-regulated status in 1999 (USDA-APHIS-BRS, 1999). The barnase protein produced in MS11 canola has an identical phenotype of male sterility to the barnase protein produced in MS8 as determined by the weight of evidence using molecular characterization of the MS11 using PCR and southern blot analysis, and phenotype

observations of the inserted DNA (AgrEvo USA Company, 1998; Bayer CropScience, 2016). The MS8 event (ACS-BNØØ3-6) was the subject of an FDA consultation in 1998, as summarized in Biotechnology Consultation BNF No. 000057, dated September 16, 1998. The male sterility restorer event (event RF3; ACS-BNØØ5-8) was also included in the FDA Consultation BNF No. 000057 (U.S. FDA 1998a,b).

The safety of the PAT proteins has been previously established (Herouet, 2005; ILSI, 2011; OECD, 1999). The safety of PAT in existing commercial transgenic crop products is supported by a permanent exemption from food and feed tolerances in all crops in the U.S. (EPA, 2007). The PAT protein expressed in MS11 canola is the same PAT protein expressed in the previously non-regulated GE canola MS8 (Bayer CropScience, 1998).

D. Potential Plant Pest and Disease Impacts

APHIS assessed data and information presented in the extension request related to the similarity of the MS11 event to Bayer antecedent canola event MS8 to determine whether potential plant pest or disease impacts are likely to result from the transformation process, from DNA sequences from plant pests, or from any other expression products, enzymes, or proteins in the MS11 event that are known or anticipated to cause disease symptoms, or to affect plant pests or diseases or plant defense responses. APHIS also assessed whether the MS11 event is more likely to have significantly increased disease and pest susceptibility as compared to the MS8 antecedent canola event. Impacts or changes in similarity to the MS8 antecedent canola event to the MS11 event were assessed to determine if they would (1) affect and/or result in significant introduction or spread of a damaging pest or disease to other plants; (2) result in the introduction, spread, and/or creation of a new disease; and/or (3) result in a significant exacerbation of a pest or disease for which APHIS has a control program.

Plant Protection and Quarantine (PPQ) is an APHIS program that safeguards agriculture and natural resources from the entry, establishment, and spread of animal and plant pests and noxious weeds into the United States; and supports trade and exports of U.S. agricultural products. PPQ responds to new introductions of plant pests to eradicate, suppress, or contain them through various programs in cooperation with state departments of agriculture and other government agencies. These may be an emergency or longer term domestic programs that target a specific pest. A variety of insect, plant disease, mollusk, nematode or weed programs exist (USDA-APHIS, 2016c); however, none specifically target pests of the MS11 event.

Because the genetic makeup and transformation of the M11 event are similar to previously deregulated Bayer antecedent canola event MS8, with the additional *barstar* gene expressed at low levels that do not alter the male sterile phenotype, no significant changes in composition are expected from the expression of genes in the MS11 event. Similarly, the MS11 event is not expected to differ from the antecedent event MS8 in its ability to harbor or transmit plant pathogens or pests and cause indirect plant pest effects on other agricultural products.

E. Potential Impacts on Non-target Organisms Beneficial to Agriculture

APHIS has previously evaluated the potential impacts on non-target organisms beneficial to agriculture that could result from the non-regulated status of Bayer antecedent MS8 canola event. The Bayer antecedent canola event was determined by APHIS to be unlikely to have an adverse effect on non-target organisms in the environment. The DNA sequences in pTHW107 (*barnase* and *bar*) used to transform the antecedent line, and the DNA sequences in pTHW118 (*barstar* and *bar*) from a second non-regulated GE canola line, encode:

- *barnase* (ribonuclease Barnase) for male sterility.
- *bar* (phosphinothricin-N-acetyltransferase) for glufosinate tolerance
- *barstar* (ribonuclease inhibitor, Barstar) for the restoration of male fertility

The exact same DNA sequences used to transform the Bayer (AgrEvo) antecedent canola MS8 event were used to transform the M11 event, and the additional *barstar* gene is expressed at low levels that do not alter the male sterile phenotype. Therefore, based on the similarity of the MS11 event to the Bayer (AgrEvo) antecedent canola MS8 event, , the unlikely impacts of non-target effects due to the genetic constructs, and the finding that the Bayer antecedent canola MS8 event was unlikely to harm non-target organisms, APHIS concludes that it is unlikely that MS11 event will have an adverse effect on non-target organisms, including those beneficial to agriculture.

F. Potential for Enhanced Weediness of the MS11 Canola

The biology of canola is well studied and cultivated canola (*Brassica napus*) is rarely weedy or persistent. However, *B. napus* can survive outside of cultivation given specific conditions, such as access to disturbed soil areas (Myers, 2006; OECD, 1997; OECD, 2012).

Brassica spp is listed on the Michigan weed list, but not specifically cultivated canola, *B. napus* (USDA-NRCS, 2013a). *Brassica napus* is listed as “introduced” in the U.S., but is not on the Federal Noxious Weed List (USDA-NRCS, 2013a).

In addition to considerations of the known biology of canola, APHIS analyzed information submitted in the petition on the antecedent MS8 canola event on a suite of agronomic characteristics and plant-disease and plant-insect interactions. This agronomic data from the field showed that the antecedent was not different than its non-transgenic comparator. The assessment concluded that the antecedent was unlikely to become a weed. In addition, in the current petition, Bayer compared their MS11 event to six non-GE conventional canola varieties as well as the non-GE parental variety (N90-740) for agronomic properties and susceptibilities to disease and pests. Bayer reported no differences in the following properties from planting to harvest: early stand count, days to flowering, days to maturity, seed yield, plant height, final stand count, sterile plants per plot, lodging resistance, pod shattering, and response to environmental conditions

including disease and pest susceptibility. This data demonstrates that MS11 was not different than its non-transgenic comparator (Bayer CropScience, 2016, p. 84). Based on the high similarity of the MS11 canola event to the antecedent canola event MS8 expressing similar proteins, the fact that the additional *barstar* gene is expressed at low levels that do not alter the male sterile phenotype, the finding that the antecedent organism was unlikely to become a weed, and the agronomic data obtained in field trials of the MS11 event, APHIS concludes that it is unlikely that MS11 event will become a weed.

G. Potential Impacts on the Weediness of Any Other Plants with which the MS11 Canola Can Interbreed

In 1999, APHIS evaluated the potential for gene introgression to occur from the antecedent Bayer (AgrEvo USA Company) canola MS8 event to sexually compatible wild relatives and considered whether transgene introgression would result in increased weediness (USDA-APHIS-BRS, 1999). APHIS is updating its analysis of the potential impacts on the weediness of any other plants with which the MS11 canola can interbreed, taking into account more recent reviews of the same species and whether or not there are new concerns or species that need to be addressed (OECD, 2016b; OECD, 2012; Tsuda, 2014).

In the earlier evaluation, five species in the U.S. were reported to hybridize with *B. napus* by open pollination: *B. rapa*, *B. nigra*, and *B. juncea* using fully fertile parents and *Raphanus raphanistrum* and *B. adpressa* (basionym *Hirschfeldia incana* L., syn. *Sinapsis incana* L. (GBIF, 2016) using a male-sterile *B. napus* parent (Scheffler and Dale, 1994). Hybrids were readily formed with *B. rapa* but had reduced fertility and dormancy relative to established wild *B. rapa*. Thus, APHIS concluded it was very unlikely that populations of *B. rapa* x *B. napus* hybrids would persist (USDA-APHIS-BRS, 1999). Hybrids were reported at very low rates with *B. nigra*, *B. juncea*; and *R. raphanistrum* and hybrids with *B. nigra* were male sterile while hybrids with the other species had low or very low fertility (OECD, 1997; AgrEvo USA Company, 1998 Appendix 2; USDA-APHIS-BRS, 1998; USDA-APHIS-BRS, 1999). The AgrEvo USA Company petition (1998) did not specifically address the weed status of the five species that *B. napus* could hybridize, perhaps because of lack of information. It should be noted that currently in the U.S., *B. napus*, *B. rapa*, *B. nigra*, *B. juncea*, *B. adpressa* and *R. raphanistrum* are listed as weeds by the Weed Science Society of America (2016) *B. rapa* appears to be the most problematic weed in cultivated crops according to recent literature (OECD, 2012; Tsuda, 2014).

OECD (2012) identified 18 species of Brassicaceae species related to *B. napus* in North America and Europe to which gene introgression from *B. napus* could be a concern and rated the potential degree of success of hybridization and gene introgression occurring with natural crossings (OECD, 2012 Table 13). The same five species related to *B. napus* discussed above as problematic in crop cultivation still exist as having the potential to

readily hybridize with *B. napus*, although new information has re-ranked their ability to do so (OECD, 2012; Tsuda, 2014). The highest degree of natural pollination was found to be with crosses between *B. napus* and *B. rapa*, while *B. napus* and *B. juncea* are considered to have the second highest crossability, but with less seed produced. The likelihood of gene introgression was rated high for both species (Scheffler and Dale 1994; OECD, 2012; Tsuda, 2014). *B. napus* x *R. raphanistrum* hybrids were created at a high rate, but with low likelihood of gene introgression and only when *B. napus* was the male-sterile parent, as reported in the earlier analysis (USDA-APHIS-BRS, 1999; OECD, 2012). The likelihood of hybridization and gene introgression in crosses with *B. nigra* was rated low, while crosses with *H. incana* were reported at higher frequency than found in the 1999 USDA analysis (USDA-APHIS-BRS, 1999), but with only a low likelihood of gene introgression (OECD 2012). Finally, there is a low likelihood of hybridization and gene introgression between *B. napus* and *B. carinata*, which has recently been introduced in North America (OECD, 2012).

Despite the potential for hybridization and transgene introgression into these sexually compatible species, APHIS previously concluded that transgene introgression from either the antecedent MS8 event or the previously deregulated RF3 event into the other species with which they can interbreed described above was unlikely to increase the weediness of those species any more than gene introgression from other canola cultivars currently available, as well as other non-transgenic, herbicide tolerant or cytoplasmic male-sterile canola cultivars. Moreover, APHIS concluded that introgression of the *barnase* transgene would most likely result in male sterility (USDA-APHIS-BRS, 1999). Although this transgene is linked to the *barstar* transgene in the MS11 event, *barstar* is not expressed in sufficient levels to reverse the male-sterile phenotype (Bayer CropScience, 2016). APHIS also previously concluded that, in agricultural settings, introgression of the transgene conferring glufosinate tolerance into one of these weedy relatives may provide a competitive advantage if glufosinate is used for weed management; however, other herbicides or mechanical means can be used to successfully control such weeds (USDA-APHIS-BRS, 1999). Despite changes in the hybridization rankings of the five problematic U.S. weedy relatives of *B. napus* that can interbreed, and the addition of *B. carinata* as a sixth relative that can interbreed, since no new genes are present in event MS11 compared to the antecedent event MS8 and the previously deregulated event RF3, APHIS reaffirms its previous conclusions.

Based on the high similarity of the MS11 canola event to the antecedent canola event MS8 expressing similar proteins, the fact that the additional *barstar* gene is expressed at low levels that do not alter the male-sterile phenotype, and the finding that gene introgression from the antecedent organism to other species with which it can interbreed was unlikely to increase the weediness of those species, APHIS concludes that it is similarly unlikely that gene introgression from MS11 event to other organism with which it can interbreed will increase their weediness.

H. Potential Changes to Agriculture or Cultivation Practices

APHIS assessed whether significant changes to agricultural or cultivation practices from the Bayer antecedent canola MS8 event are likely to impact plant diseases or pests or their management, including any APHIS control programs. This includes consideration of any changes in pesticide applications, tillage, irrigation, harvesting, etc. as they relate to plant pests and diseases.

APHIS did not identify any significant changes to agricultural or cultivation practices (e.g. pesticide applications, tillage, irrigation, harvesting, rotations, management of volunteers, etc.) from the Bayer antecedent canola MS8 event and concluded that no impact on plant diseases or pests or their management is likely to occur. Based on the similarity of the MS11 event to the Bayer antecedent canola MS8 event expressing similar proteins and the fact that the additional *barstar* gene is expressed at low levels that do not alter the male sterile phenotype, APHIS concludes that it is unlikely that any significant changes to agriculture or cultivation practices would be associated with the MS11 event and therefore no impact on plant diseases or pests of their management are likely to occur.

I. Potential Impacts from Transfer of Genetic Information to Organisms with which the MS11 Canola Cannot Interbreed

APHIS has previously examined the potential for the introduced genes in the Bayer antecedent canola MS8 event, *barnase* (ribonuclease barnase) for male sterility, and *bar* (phosphinothricin-N-acetyltransferase) for glufosinate tolerance to be horizontally transferred without sexual reproduction to other organisms and whether such an event could lead directly or indirectly to disease, damage, injury or harm to plants, including the creation of new or more virulent pests, pathogens, or parasitic plants (USDA-APHIS-BRS, 1999). APHIS also assessed the potential for the other gene in the MS11 event, *barstar* (ribonuclease inhibitor, for the restoration of male fertility) for the same attributes listed above when it assessed event RF3 (USDA-APHIS-BRS, 1999). The horizontal gene transfer between unrelated organisms is one of the most intensively studied fields in the biosciences 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 horizontal gene transfer (HGT) from genetically engineered organisms to another organism without reproduction or human intervention were recently reviewed (Keese, 2008). Mechanisms of HGT include conjugation, transformation and transduction, and other diverse mechanisms of DNA and RNA uptake and recombination and rearrangement, most notably through viruses and mobile genetic elements. APHIS has previously reviewed the potential for horizontal gene transfer from GE canola to bacteria, fungi, invertebrates, viruses, and parasitic plants (USDA-APHIS-BRS, 1999).

APHIS previously concluded that HGT of the inserted genetic material from the Bayer antecedent canola MS8 event and the RF3 event to other organisms is highly unlikely, and is not expected to lead directly or indirectly to disease, damage, injury or harm to plants, including the creation of new or more virulent pests, pathogens, or parasitic

plants. Therefore, APHIS concludes that HGT from the MS11 event to other organisms is also highly unlikely.

J. Conclusion

APHIS has reviewed the information submitted in the extension request, supporting documents, and other relevant information to assess the similarity of plant pest risk of the MS11 event compared to the Bayer antecedent canola MS8 event. APHIS concludes that the MS11 event is **no more likely** to pose a plant pest risk than the previously deregulated Bayer antecedent canola MS8 event.

K. References

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L. Similarity Table

Description		Extension Request MS11 Petition 16-235-01p	Antecedent MS8 Petition 98-278-01p	Comments
Organism		***Brassica napus* (Canola)**	***Brassica napus* (Canola)**	**Drakkar variety was used for the antecedent MS8 event and the N90-740 variety was used for the MS11 event**
Phenotype		**Glufosinate-ammonium tolerant; male-sterile**	**Glufosinate-ammonium tolerant; male-sterile**	
Genotype		**Construct pTC0113**	C**onstruct pTHW107**	
	Bar cassette	*PSsuAra* promoter from *Arabidopsis thaliana* *bar* gene from *Streptomyces hyqroscopicus* *3'g7* terminator from *Agrobacterium tumefaciens*	*PSsuAt* promoter from *Arabidopsis thaliana* *bar* gene from *Streptomyces hyqroscopicus* *3'g7* terminator from *Agrobacterium tumefaciens*	The promoter genes, *PSsuAra* and *PSsuAt*, are synonyms for the same gene. The same genes, promoters, and spacers are used in the MS11 event that were used in the MS8 event.
	Barnase cassette	*PTA29* promoter: *Nicotiana tabacum* *barnase: Bacillus amyloliquefaciens* *3' barnase: Bacillus amyloliquefaciens* *3'nos*: *Agrobacterium tumefaciens*	*PTA29* promoter from *Nicotiana tabacum* *barnase: Bacillus amyloliquefaciens* *3' barnase: Bacillus amyloliquefaciens* *3'nos: Agrobacterium tumefaciens*	

Description		**Extension Request MS11 Petition 16-235-01p**	**Antecedent MS8 Petition 98-278-01p**	**Comments**
Organism		***Brassica napus* (Canola)**	***Brassica napus* (Canola)**	**Drakkar variety was used to transform the antecedent MS8 and the N90-740 variety was used for the MS11 event**
Genotype	Barstar cassette	**Construct pTHW118** *pNos*: *Agrobacterium tumefaciens* *barstar: Bacillus amyloliquefaciens* *3'g7*: *Agrobacterium tumefaciens*	*N/A*	MS11 event has the exact coding sequences found in the previously non-regulated GE canola event RF3. This gene cassette is only present to facilitate *Agrobacterium*-mediated transformation
Transformation Method		*Agrobacterium tumefaciens*–mediated	*Agrobacterium tumefaciens*–mediated	Same
Insert and Copy Number		Single intact insertions	Single intact insertions	Same
Compositional analysis		No notice of voluntary FDA consultation has been reported to USDA-APHIS-BRS to date.	Compositionally equivalent to conventional *Brassica napus* (Canola) according to the voluntary consultation with FDA signed on Sept 16, 1998	Not confirmed
Backbone Absent		Yes	Yes	Same
Mechanism of Action		Male sterility by expression of Barnase in the tapetum cells; Glufosinate-ammonium tolerance by expression of PAT/bar. ----------	Male sterility by expression of Barnase in the tapetum cells; Glufosinate-ammonium tolerance by expression of PAT/bar ----------	The MS11 event contains the similar gene constructs and mechanism of action as the antecedent MS8 event.

	bar*: Streptomyces hyqroscopicus* coding sequence of the phosphinothricin acetyltransferase ---------- Barnase*: Bacillus amyloliquefaciens* results in lack of viable pollen and male sterility	bar*: Streptomyces hyqroscopicus* coding sequence of the phosphinothricin acetyltransferase ---------- Barnase*: Bacillus amyloliquefaciens* results in lack of viable pollen and male sterility	
Date of antecedent EA/ EIS	N/A	Granted non-regulated status on March 31, 1999	

Description	Extension Request MS11 Petition 16-235-01p	Antecedent MS8 Petition 98-278-01p	Comments
Organism	***Brassica napus* (Canola)**	***Brassica napus* (Canola)**	
Disease and pest susceptibilities	Similar as antecedent	Unlikely to change disease and pest susceptibilities	
Impacts on beneficial non-targets	Similar as antecedent	Unlikely to impact beneficial non-target organisms	
Enhanced weediness	Similar as antecedent	Unlikely to enhance weediness	
Enhanced weediness of relatives	Similar as antecedent	Unlikely to enhance weediness of relatives	
Changes to agriculture or cultivation practices	Similar as antecedent	Unlikely to change agriculture or cultivation practices	
Horizontal Gene Transfer	Similar as antecedent	Unlikely to affect the probability of horizontal gene transfer	
Plant Pest Risk	Similar as antecedent	Unlikely to pose a plant pest risk	