DEPARTMENT OF AGRICULTURE

Animal and Plant Health Inspection Service

[Docket No. 98–114–2]

AgrEvo USA Co.; Availability of Determination of Nonregulated Status for Canola Genetically Engineered for Male Sterility, Fertility Restoration, and Glufosinate Herbicide Tolerance

AGENCY: Animal and Plant Health Inspection Service, USDA.

ACTION: Notice.

SUMMARY: We are advising the public of our determination that certain canola transformation events developed by AgrEvo USA Company, which have been genetically engineered for male sterility, fertility restoration, and tolerance to the herbicide glufosinate, are no longer considered regulated articles under our regulations governing the introduction of certain genetically engineered organisms. Our determination is based on our evaluation of data submitted by AgrEvo USA Company in its petition for a determination of nonregulated status and on our analysis of other scientific data. This notice also announces the availability of our written determination document and its associated environmental assessment and finding of no significant impact.

EFFECTIVE DATE: March 22, 1999.

ADDRESSES: The determination, an environmental assessment and finding of no significant impact, and the petition may be inspected at USDA, room 1141, South Building, 14th Street and Independence Avenue SW., Washington, DC, between 8 a.m. and 4:30 p.m., Monday through Friday, except holidays. Persons wishing to inspect those documents are asked to call in advance of visiting at (202) 690–2817 to facilitate entry into the reading room.

FOR FURTHER INFORMATION CONTACT: Dr. Susan Koehler, Biotechnology and Biological Analysis, PPQ, APHIS, Suite 5B05, 4700 River Road Unit 147, Riverdale, MD 20737–1236; (301) 734–4886. To obtain a copy of the determination or the environmental assessment and finding of no significant impact, contact Ms. Kay Peterson at

(301) 734–4885; e-mail: kay.peterson@usda.gov.

SUPPLEMENTARY INFORMATION:

Background

On October 5, 1998, the Animal and Plant Health Inspection Service (APHIS) received a petition (APHIS Petition No. 98–278–01p) from AgrEvo USA Company (AgrEvo) of Wilmington, DE, seeking a determination that canola (*Brassica napus* L.) designated as In Vigor® Hybrid Canola Transformation Events MS8 and RF3 (transformation events), which have been genetically engineered for male sterility (MS8), fertility restoration (RF3), and tolerance to the herbicide glufosinate (both MS8 and RF3), do not present a plant pest risk and, therefore, are not regulated articles under APHIS' regulations in 7 CFR part 340.

On December 8, 1998, APHIS published a notice in the **Federal Register** (63 FR 67643–67644, Docket No. 98–114–1) announcing that the AgrEvo petition had been received and was available for public review. The notice also discussed the role of APHIS, the Environmental Protection Agency, and the Food and Drug Administration in regulating the subject canola transformation events and food products derived from them. In the notice, APHIS solicited written comments from the public as to whether these canola transformation events posed a plant pest risk. The comments were to have been received by APHIS on or before February 8, 1999. APHIS received no comments on the subject petition during the designated 60-day comment period.

Analysis

The subject transformation events have been genetically engineered to contain a *barnase* gene (MS8) for male sterility or a *barstar* gene (RF3) for fertility restoration. The *barnase* gene expresses a ribonuclease that blocks pollen development and results in a male sterile plant, and the *barstar* gene encodes a specific inhibitor of this ribonuclease and restores fertility. The *barnase* and *barstar* genes were derived from *Bacillus amyloliquefaciens*, and are linked in the subject transformation events to the *bar* gene derived from *Streptomyces hygroscopicus*. The *bar* gene encodes the enzyme phosphinothricin-N-acetyltransferase (PAT), which confers tolerance to the herbicide glufosinate. The herbicide tolerance trait allows for selection of plants carrying the linked genes for pollination control during breeding and for tolerance to the herbicide during commercial cultivation. Expression of the added genes is controlled in part by gene sequences derived from *Arabidopsis thaliana*, *Nicotiana tabacum*, and the plant pathogen *Agrobacterium tumefaciens*. The *A. tumefaciens* method was used to transfer the added genes into the parental canola variety, Drakkar.

Canola transformation events MS8, RF3, and their hybrid combination MS8/RF3 have been considered regulated articles under APHIS' regulations in 7 CFR part 340 because they contain gene sequences derived from a plant pathogen. However, evaluation of field data reports from field tests of these canola transformation events conducted under APHIS permits and notifications since 1997 indicates that there were no deleterious effects on plants, nontarget organisms, or the environment as a result of the environmental release of the subject canola transformation events.

Determination

Based on its analysis of the data submitted by AgrEvo and a review of other scientific data and field tests of the subject canola, APHIS has determined that canola transformation events MS8, RF3, and their hybrid combination MS8/RF3: (1) Exhibit no plant pathogenic properties; (2) are no more likely to become weeds than canola developed by traditional breeding techniques and are unlikely to increase the weediness potential for any other cultivated or wild species with which they can interbreed; (3) will not cause damage to raw or processed agricultural commodities; (4) will not harm threatened or endangered species or other organisms, such as bees, that are beneficial to agriculture; and (5) are unlikely to have any significant adverse impact on agricultural practices. Therefore, APHIS has concluded that the subject canola transformation events and any progeny derived from hybrid crosses with other canola varieties will be as safe to grow as canola in breeding programs that are not subject to regulation under 7 CFR part 340.

The effect of this determination is that AgrEvo's canola transformation events MS8, RF3, and their hybrid combination MS8/RF3 are no longer considered regulated articles under APHIS' regulations in 7 CFR part 340. Therefore, the requirements pertaining to regulated articles under those regulations no longer apply to the subject canola transformation events or their progeny. However, importation of these canola transformation events or seeds capable of propagation are still subject to the restrictions found in APHIS' foreign quarantine notices in 7 CFR part 319.

National Environmental Policy Act

An environmental assessment (EA) has been prepared to examine the potential environmental impacts associated with this determination. The EA was prepared in accordance with: (1) The National Environmental Policy Act of 1969 (NEPA), as amended (42 U.S.C. 4321 *et seq.*), (2) regulations of the Council on Environmental Quality for implementing the procedural provisions of NEPA (40 CFR parts 1500–1508), (3) USDA regulations implementing NEPA (7 CFR part 1b), and (4) APHIS' NEPA implementing Procedures (7 CFR part 372). Based on that EA, APHIS has reached a finding of no significant impact (FONSI) with regard to its determination that AgrEvo's canola transformation events MS8, RF3, and their hybrid combination MS8/RF3 and lines developed from them are no longer regulated articles under its regulations in 7 CFR part 340. Copies of the EA and the FONSI are available upon request from the individual listed under **FOR FURTHER INFORMATION CONTACT.**

Done in Washington, DC, this 24th day of March 1999.

Craig A. Reed,

Administrator, Animal and Plant Health Inspection Service.

[FR Doc. 99–7803 Filed 3–30–99; 8:45 am]

BILLING CODE 3410–34–P

Response to AgrEvo Petition 98-278-01p for Determination of Nonregulated Status for Canola Transformation Events MS8 and RF3 Genetically Engineered for Pollination Control and Tolerance to Glufosinate Herbicide

Finding of No Significant Impact

March 1999

The Animal and Plant Health Inspection Service (APHIS), United States Department of Agriculture, has prepared an environmental assessment prior to issuing a determination in response to a petition (APHIS Number 98-278-01p) received from AgrEvo USA Company regarding the status of canola transformation events MS8 and RF3 under APHIS regulations at 7 CFR Part 340. MS8 and RF3 canola are genetically engineered for male sterility and restoration of male fertility, respectively, and both transformation events are genetically engineered for tolerance to the herbicide glufosinate-ammonium. The purpose of this pollination control system is to enable the production of pure hybrid canola varieties.

APHIS has conducted an extensive review of the petition and supporting documentation, as well as other relevant scientific information. A thorough evaluation of the potential for significant impact to the human environment has brought APHIS to a Finding of No Significant Impact (FONSI). This conclusion is based on our analysis that MS8 and RF3 canola: (1) exhibit no plant pathogenic properties either as a result of the transformation process itself or from the insertion and expression of new genetic material conferring the herbicide tolerance and pollination control traits; (2) are no more likely to become weeds, or increase the weediness potential or effect biodiversity of sexually compatible relatives, any more than commercially available canola varieties; (3) will not cause damage to raw or processed agricultural commodities; (4) will not harm organisms beneficial to plants (e.g., bees and earthworms), or threatened or endangered species; (5) are unlikely to have any significant adverse impact on agricultural practices.

APHIS has also concluded that there is a reasonable certainty that new progeny varieties bred from MS8 and/or RF3 will not exhibit new plant pest properties, i.e., properties substantially different from any observed for MS8 or RF3 canola, or those observed for traditionally bred canola.

In conjunction with the FONSI, APHIS has made the determination that MS8 and RF3 canola transformation events and progeny derived from either of these have no potential to pose a plant pest risk, and are, therefore, no longer regulated articles under regulations at 7 CFR part 340.

for Mary S. Heal

Sally L. McCammon
Acting Assistant Director
Scientific Services
Plant Protection and Quarantine
Animal and Plant Health Inspection Service
U.S. Department of Agriculture
Date: MAR 22 1999

ENVIRONMENTAL ASSESSMENT

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I. SUMMARY

The Animal and Plant Health Inspection Service (APHIS), U.S. Department of Agriculture (USDA), has prepared an Environmental Assessment (EA) in response to a petition (APHIS Number 98-278-01p) from AgrEvo USA Company (AgrEvo) regarding canola transformation events MS8 and RF3. AgrEvo seeks a determination that these canola transformation events do not present a plant pest risk and should therefore no longer be regulated articles under regulations at 7 CFR Part 340.

The subject canola transformation events were genetically engineered for male sterility (MS8), restoration of male fertility (RF3), and tolerance to the herbicide glufosinate (both MS8 and RF3), to enable the production of pure hybrid canola varieties by the use of a pollination control system. The genes controlling pollination, *barnase* and *barstar*, were derived from the bacterium *Bacillus amyloliquefaciens*. The gene controlling glufosinate tolerance, *bar*, was derived from the bacterium *Streptomyces hygroscopicus*. These canola have been considered regulated articles because a plant pest, *Agrobacterium*, was used as a vector for the insertion of these genes into these canola and as a donor of certain sequences used to regulate expression of these genes.

Field trials of MS8 and RF3 canola and their progeny have been conducted under permits and notification acknowledged by APHIS according to regulations at 7 CFR Part 340. Performance standards and conditions for such field trials require that the regulated article and its offspring must not persist in the environment after completion of the test. This Environmental Assessment (EA) specifically addresses the potential for impacts to the human environment through use in agriculture of MS8 and RF3 canola or progeny derived from them following a determination of nonregulated status by APHIS under 7 CFR Part 340.

II. PROPOSED ACTION - Description and Statement of Purpose and Need.

APHIS Regulatory Authority for the Introduction of MS8 and RF3 Canola. The USDA/APHIS has recieved a petition (98-278-01p) submitted by AgrEvo for a determination of nonregulated status of MS8 and RF3 canola and their progeny. The purpose of this Environmental Assessment (EA) is to ascertain whether the proposed approval of this petition, which would allow for the unconfined introduction into the U.S. or its territories of these canola, would have a significant impact on the environment. This petition was submitted pursuant to regulations codified in 7 CFR Part 340. These regulations, entitled "Introduction of Organisms and Products Altered or Produced Through Genetic Engineering Which Are Plant Pests or Which There is Reason to Believe Are Plant Pests" govern the introduction (importation, interstate

movement, or release into the environment or any attempt thereat) of certain genetically engineered organisms and products.

MS8 and RF3 canola have been genetically engineered to express a *bar* gene derived from the bacterium *Streptomyces hygroscopicus*. The *bar* gene encodes the enzyme phosphinothricin-N-acetyltransferase (PAT) that confers tolerance to the post-emergence, broad-spectrum herbicide glufosinate-ammonium in MS8 and RF3 canola. In addition, MS8 and RF3 have been engineered with genes to control pollination and allow for the production of hybrids. MS8 has been engineered to express a ribonuclease encoded by the *barnase* gene derived from the bacterium *Bacillus amyloliquefaciens*. The ribonuclease blocks pollen development and results in male sterility in MS8 canola or progeny containing the gene. RF3 has been engineered to express a specific inhibitor of this ribonuclease encoded by the *barstar* gene, which is also derived from *B. amyloliquefaciens*. The ribonuclease inhibitor restores male fertility in plants containing the *barnase* gene. Thus male fertile canola plants such as RF3 that express the *barstar* gene can be used in controlled pollinations of male sterile canola plants such as MS8 that contain the *barnase* gene to produce hybrid progeny with restored male fertility. MS8 and RF3 canola have been considered "regulated articles" under 7 CFR Part 340 because the plant pathogen *Agrobacterium tumefaciens* was used as a transformation vector agent and as a source of noncoding sequences used to regulate the expression of inserted genes.

These canola have been extensively field tested in Canada, Europe, and the United States. Field testing in the U. S. has been conducted since 1997 only under conditions of physical and reproductive confinement as authorized by USDA permits (97-035-05r, 98-119-01r) and notifications (98-064-38n, 98-064-35n, 98-064-33n, 98-168-04n, 98-064-31n) according to APHIS regulations at 7 CFR Part 340. Prior to issuing a permit or notification for a field release, APHIS analyzes the potential impacts associated with the proposed introduction. AgrEvo has submitted field data reports for field tests conducted in the U.S. and data from the Canadian and European trials. These reports give information on the biological and agronomic characteristics of the plant, oil and seed quality, and any potential adverse effects on plants, nontarget organisms, or the environment associated with the field trial.

An organism is not subject to the regulatory requirements of 7 CFR Part 340 when it is demonstrated not to present a plant pest risk. Section 340.6 of the regulations, entitled "Petition for determination of nonregulated status," provides that a person may petition the Agency to evaluate submitted data and determine whether a particular regulated article does not present a risk of introduction or dissemination of a plant pest. If a determination of nonregulated status is made, the petition would be granted, thereby allowing for unregulated introduction of the article in question. Permits and notifications under those regulations would then no longer be required from APHIS for field testing, importation, or interstate movement of that article or its progeny. Normal

agronomic practices with it, e.g., cultivation, propagation, movement, and cross-breeding, could then be conducted without APHIS approval.

Prior to issuing a determination of nonregulated status, APHIS considers regulatory alternatives and evaluates the potential for significant impact to the human environment, in accordance with regulations and procedures implementing the National Environmental Policy Act (NEPA), as amended (42 U.S.C. 4321 *et seq.*); 40 CFR Parts 1500-1508; 7 CFR Part 1b; 7 CFR Part 372.

Food and Drug Administration (FDA) and Environmental Protection Agency (EPA) Regulatory Authority over MS8 and RF3 Canola. The FDA has authority to ensure the safety and wholesomeness of all food(s). The FDA policy statement concerning the regulation of foods derived from new plant varieties, including genetically engineered plants, was published in the Federal Register on May 29, 1992 (57 FR 22984-23005). Regulatory oversight for the safety of any food or feed products derived from MS8 and RF3 canola is under the jurisdiction of the FDA. FDA has granted a finding of 'No Concern' for the subject canola tranformation events in September, 1998, (please see the FDA Home Page at the following URL: (http://vm.cfsan.fda.gov/~lrd/biocon.html).

The EPA is responsible for the regulation of pesticides under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) as amended, (7 U.S.C. 136 *et seq.*). FIFRA requires that all pesticides, including herbicides, be registered prior to distribution or sale, unless exempt by EPA regulation. Under the Federal Food, Drug, and Cosmetic Act (FFDCA), as amended (21 U.S.C. 301 *et seq.*), pesticides added to (or contained in) raw agricultural commodities generally are considered to be unsafe unless a tolerance or exemption from tolerance has been established. Residue tolerances for pesticides are established by EPA under the FFDCA, and the FDA enforces those tolerances. Full registration and tolerance establishment for use of glufosinate-ammonium herbicide Liberty® on glufosinate-tolerant canola is pending with the EPA. The tolerance extension was announced by the EPA in the Federal Register on October 8, 1997 (62 FR 52544-52552) (please see the EPA Federal Register notice at the following URL: (http://www.epa.gov/docs/fedrgstr/EPA-PEST/1997/October/Day-08/p26537.htm).

III. ALTERNATIVES

In the course of preparing the environmental assessment for this petition, APHIS considered the following two alternatives: (1) deny the petition, so that MS8 and RF3 canola would continue to be regulated under 7 CFR Part 340; and (2) approve the petition, so that permits or notifications would no longer be required from APHIS under

7 CFR Part 340 for these canola tranformation events or progeny derived from them when introduced or grown in the United States and its territories.

IV. AFFECTED ENVIRONMENT AND POTENTIAL ENVIRONMENTAL IMPACTS

If APHIS denies the petition, MS8 and RF3 canola and progeny derived from either of these would continue to be regulated by APHIS under 7 CFR Part 340. Interstate movement, certain importations, and environmental releases of these canola could only be conducted under permits or notifications approved by APHIS that impose conditions of physical or reproductive confinement to prohibit persistence of these canola or their progeny in the environment. For example, to prevent out-crossing to sexually compatible species and persistence of any offspring, most canola field trials conducted under 7 CFR Part 340 require an isolation distance of 660 ft. from other commercial canola, control of sexually compatible wild or weedy relatives around the release site, strict harvesting measures, and post-harvest monitoring and termination treatments to control volunteers from the transgenic canola. AgrEvo would not be able to sell seed from these canola (or their progeny) to farmers for planting unless the farmers were able and willing to meet the conditions of the permit or notification. Farmers who grow canola for its oil and meal would find such conditions difficult, if not impossible, to meet. Denying the petition would have the effect of denying American farmers the benefit of hybrid canola seed that could be produced from MS8 and RF3 canola.

The remainder of this EA addresses potential environmental impacts from a determination that MS8 and RF3 canola or progeny derived from either of these should no longer be considered regulated articles under APHIS regulations at 7 CFR Part 340. These would be potential impacts that might be associated with cultivation and normal use in agriculture of MS8 and RF3 canola, and progeny derived from either of these, without APHIS imposed conditions of physical or reproductive confinement from other sexually compatible plants. Additional technical information is included in the determination document appended to this EA (Appendix A.), and incorporated by reference. This includes further discussion of the biology, taxonomy, cultivation, and sexual reproduction and outcrossing potential of canola as well as of the genetic components inserted into MS8 and RF3 canola, and the analyses that lead APHIS to a conclusion that these canola have no potential to pose a plant pest risk.

Potential for the introduced genes, their products, and the added regulatory sequences controlling their expression to cause plant disease. MS8 and RF3 canola are considered regulated articles because the plant pathogen, *A. tumefaciens* (the causal agent of a tumor-inducing, crown gall disease), was used as a vector in the transformation process and as a donor for genetic material inserted into these plants. Because the genes that cause crown gall disease were removed from the tumor-inducing (Ti)- plasmid in *A. tumefaciens*, the transformed plants do not develop crown gall

disease. Furthermore, initial transformed tissue was treated with an appropriate antibiotic to eliminate *Agrobacterium*; and no crown gall symptoms were reported in these canola by AgrEvo under field conditions. The specific DNA sequences from the plant pest *Agrobacterium* which were inserted into MS8 and RF3 canola cannot incite disease or result in the production of an infectious agent. Furthermore, AgrEvo provides evidence that expression of the introduced genes does not result in disease symptoms or an increase in susceptibility to diseases.

Potential impacts based on weediness potential of MS8 and RF3 canola relative to traditionally bred canola. Almost all definitions of weediness stress as core attributes the undesirable nature of weeds from the point of view of humans; from this core, individual definitions differ in approach and emphasis (Baker, 1965; de Wet and Harlan, 1975; Muenscher, 1980). In further analysis of weediness, Baker (1965) listed 12 common weed attributes which can be used as an imperfect guide to the likelihood that a plant will behave as a weed. Keeler (1989) and Tiedje et al. (1989) have adapted and analyzed Baker's list to develop admittedly imperfect guides to the weediness potential of transgenic plants; both authors emphasize the importance of looking at the parent plant and the nature of the specific genetic changes.

Despite its ability to volunteer, escape from cultivated fields, and form temporary occasional populations, the parent plant in this petition, *Brassica napus*, is not a serious weed under conditions found in the United States. *B. napus* is listed as a weed in Weed Science Society of America (1992). The comprehensive world list of Holm et al. (1991) does not list it as a serious or principal weed anywhere in the world; they do, however, give two listings as a common weed: one in Finland and one in Kenya. *B. napus* is mentioned as an "occasional weed" by Munz (1968), and "sometimes escaped" by Bailey (1949). AgrEvo has submitted substantial evidence to indicate the lack of weedy nature of MS8 and RF3 canola and their hybrids, and for other glufosinate-tolerant canola transformation events under agricultural conditions. Field observations indicate that seed germination and dormancy, seed production, pest and disease resistance characteristics, time to flowering, and sensitivity to herbicides other than glufosinate-ammonium are the same for MS8/RF3 hybrids as for nontransgenic canola.

There is no reason to believe that the new traits engineered into MS8 and RF3 canola would by themselves, cause these canola to be more weedy. These genetic alterations do not result in characteristics commonly observed in many of the world's worst weeds (Baker, 1965). Other glufosinate-ammonium tolerant canola deregulated by APHIS exhibits no increased weediness potential (USDA, 1998). As previously noted, glufosinate tolerance is unlikely to increase weediness of canola unless glufosinate is the only alternative for control of the plant. Such an alteration, because it does not confer any pest resistance or alter reproductive biology or change any physiology related to survival, does not confer a competitive advantage favoring the canola plants over unmodified varieties. Consideration of supporting data on other glufosinate-tolerant

canola also leads APHIS to believe that glufosinate tolerance will not lead to increased weediness. To increase weediness of the canola plant there would have to be selection pressure on glufosinate tolerant canola (Tiedje et al., 1989; Office of Technology Assessment, 1988). Moreover, AgrEvo presents evidence that MS8 and RF3 canola are still susceptible to other herbicides that control related mustards (e.g. glyphosate, phenoxys, and sulfonylureas). The traits controlling pollination in MS8 and RF3 canola are not expected to increase the weediness potential of canola, and in fact male sterility would provide a competitive disadvantage.

Potential impacts from gene introgression from MS8 and RF3 canola into wild relatives. Whereas intra-specific crosses between *B. napus* cultivars occur readily, inter-specific crosses between *B. napus* and related species occur with varying degrees of success and are influenced greatly by the direction of the cross. An analysis of the potential for related species to hybridize with *B. napus* under field conditions (documented in Appendix A) has led APHIS to conclude that the potential would exist for transgene introgression from MS8 or RF3 or its hybrid to occur at a relatively low to moderate rate into *B. rapa* L. (=*B. campestris* L.), and at extremely low rates for *B. juncea*; *B. adpressa* , syn. *Herschfeldia incana* (hoary mustard); *B. nigra*; and *R. raphanistrum* (wild radish). All of these species are found in the major canola producing states of North Dakota, Minnesota, Montana, Idaho, Washington, and Georgia. Of these species, *B. juncea, B. nigra, and B. rapa* to some degree are agricultural weeds, sometimes serious, in much of the United States (Gleason, 1952; Slife et al., 1960; Reed, 1970; Muenscher, 1980). Reduced dormancy of *B. rapa* x *B. napus* hybrids relative to the persistent wild *B. rapa*, coupled with the reduced fertility of the inter-specific hybrid makes it very unlikely that populations of these hybrids will persist. There is a small chance that hybrids could backcross to wild *B. rapa* and thereby transfer the transgenes to wild populations (Crawley et al. 1993). Introgression into these other *Brassica* species and wild radish will be limited due to effects such as reduced fertility of the hybrids, triploidy, and chromosome incompatibilities, depending on the species.

Since MS8 and RF3 canola and their hybrids do not exhibit weedy characteristics or have any fitness advantage as a result of the transgenes, and due to the lack of selection pressure for these expressed traits outside of cultivation, transgene introgression into the sexually compatible relatives described above is unlikely to increase their weediness or impact their biodiversity anymore than would gene introgression from other canola cultivars currently available, including other nontransgenic, herbicide tolerant or cytoplasmic male sterile canola cultivars. The *barnase* and *barstar* genes would be expected to segregate independently of each other. Introgression of the *barnase* transgene in the absence of the *barstar* gene would most likely result in male sterility which would further limit gene introgression. 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.

Potential impact on nontarget organisms, including beneficial organisms such as bees and earthworms, and endangered or threatened species. There is no reason to believe that deleterious effects on beneficial organisms could result from the cultivation of MS8 or RF3 canola or their hybrid. First the trait controlling male sterility affects only anther and pollen development; flower nectaries, which provide a source of nutrients for pollinators, develop normally, and the flowers do not show a greater tendency towards bud abortion. The RF3 plants and the hybrids have normal flower morphology, fertility, and attractiveness to insect pollinators. Normal insect activity was observed on all these plants. The new transgene proteins expressed in the transgenic canola plants were derived from common soil bacterium, and ribonucleases and ribonuclease inhibitors are common in bacteria and plants. Therefore, the same or similar proteins are normal parts of the diets of animals, humans and insects. Other glufosinate tolerant canola transformation events have not been shown to be harmful to beneficial organisms or threatened and endangered species (USDA, 1998). Knowledge of the mode of action, and the lack of known toxicity for the newly expressed proteins suggest no potential for deleterious effects on beneficial organisms such as bees and earthworms. MS8 and RF3 canola and their hybrid do not contain elevated levels of toxic oils, and therefore, insects that may feed on these canola will not be unduly affected in their ability to reproduce or function normally after feeding. Results of trials in the United States, Canada, and Europe do not reveal any noticeable adverse effects on beneficial organisms. Common insects that feed on canola are not on the list of threatened and endangered species. APHIS concludes that the unconfined cultivation of MS8 and RF3 canola will not have deleterious effects, either directly or indirectly on organisms that are recognized as beneficial to agriculture or on threatened and endangered species.

Potential damage to processed agricultural commodities. The FDA has issued a finding of 'No Concern' for these canola transformation events in September 1998, and the use of these canola for food and feed purposes has also been granted by Canada. Erucic acid and glucosinolates are the only two toxicants known in rapeseed. MS8 and RF3 canola has been developed from low erucic acid and low glucosinolate canola varieties, and these transformation events were selected, in part, for normal oil and seed quality. AgrEvo confirmed that the erucic acid level was not higher than that expected for the canola variety from which MS8 and RF3 canola were developed. As such, MS8 and RF3 canola should not present any concerns as far as toxicological properties of canola. APHIS notes that Agriculture and Agri-Food Canada (1996) concludes that AgrEvo provided data which demonstrated that the nutritional composition of the whole seed, processed meal or oil derived from MS8, RF3, and their hybrid is substantially equivalent to conventional canola varieties. APHIS concludes that MS8,

RF3 and their hybrid should not have a direct or indirect plant pest effect on any processed commodity.

Potential impacts on biodiversity. Our analysis determined that genetically engineered MS8 and RF3 canola and their progeny are no more likely to become weeds, or increase the weediness potential of any other cultivated plant or native wild species with which they can interbreed, any more than other commerical canola developed by traditional breeding techniques. They will not harm threatened and endangered species and non-target organisms, they are still attractive to pollinators, and the nutritional composition and toxicological properties of their seed products are within normal limits. APHIS therefore concludes that there unlikely to be a significant impact on biodiversity from the proposed action.

Potential impacts on agricultural and cultivation practices. APHIS has previously issued determinations of nonregulated status to other genetically-engineered glufosinate-tolerant canola (USDA, 1998) and corn engineered for male sterility (USDA, 1996) with similar genetic constructs as those used in MS8 and RF3 canola. APHIS is unaware of any adverse impacts on agricultural practices associated with the cultivation of these. Male-sterile oilseed rape plants are already used to some extent to develop hybrids. The pollination control system engineered into MS8 and RF3 canola, along with the glufosinate-tolerance trait, is expected to lead to a more efficient system for producing hybrid oilseed rape. F1 hybrids of canola are estimated to yield 20-25% more seeds and are more uniform than the best open-pollinated varieties.

Based on the APHIS analysis, there is unlikely to be any significant adverse impact on agricultural practices associated with the use of MS8 and RF3 canola. However, it is of concern that there is a likelihood of canola volunteers possessing a combination of two different herbicide resistance genes and how such volunteers would be managed by growers. APHIS has deregulated other canola engineered for resistance to two different broad-spectrum post-emergent herbicides, glufosinate (USDA, 1998) and glyphosate (USDA, 1999). These canola are still sensitive to other herbicides, and information has been provided regarding the use in different crops of alternative herbicides which could be used to control *Brassica* volunteers or weeds should they obtain, through crossing, resistance to glufosinate and/or other herbicides with different modes of action.

Consideration of potential environmental impacts outside the United States associated with the proposed action. APHIS has also considered potential environmental impacts outside the United States and its territories associated with the proposed determination of nonregulated status of MS8 and RF3 canola, and progeny derived from them. This determination would allow for cultivation, interstate movement and importation into the United States and its territories without an APHIS permit or notification under 7 CFR Part 340. It does <u>not</u>, however, release the

developer from its obligation to obtain any other necessary approvals for pesticide use on these canola or for their intentional movement in international trade. Canada is a major producer of canola, and they have already granted approval for environmental release, food and feed use of these canola. Approval to market MS8 and RF3 canola in the European Union (EU) has been requested, but is pending. Several factors contribute to the conclusion that there should be no impacts abroad from cultivation of these canola lines or their progeny.

Any international traffic in the canolas subject to this determination would be fully subject to national and regional phytosanitary standards promulgated under the International Plant Protection Convention (IPPC). The IPPC has set a standard for the reciprocal acceptance of phytosanitary certification among the nations that have signed or acceded to the Convention (106 countries as of 1999). The treaty, now administered by a Secretariat housed with the Food and Agriculture Organization in Rome, came into force on April 3, 1952, and establishes standards to facilitate the safe movement of plant materials across international boundaries. Plant biotechnology products are fully subject to national legislation and regulations, or regional standards and guidelines promulgated under the IPPC. The vast majority of IPPC signatories have promulgated, and are now administering, such legislation or guidelines. The IPPC has also led to the creation of Regional Plant Protection Organizations (RPPOs) to facilitate regional harmonization of phytosanitary standards.

Issues that may relate to commercialization of particular agricultural commodities produced through biotechnology are being addressed in international fora. APHIS has played a role in working toward harmonization of biosafety guidelines and regulations included within the RPPO for our region, the North American Plant Protection Organization (NAPPO), which includes Mexico, Canada, and the United States. NAPPO's Biotechnology Panel advises NAPPO on biotechnology issues as they relate to plant protection. APHIS participates regularly in biotechnology policy discussions at fora sponsored by the EU and the Organization for Economic Cooperation and Development. In addition, APHIS periodically holds bilateral or quadrilateral discussions on biotechnology regulatory issues with other countries, most often Canada, Mexico, and Argentina. APHIS also acts as a consultant for the development of biotechnology guidelines and regulations, and has interacted with governments around the world in this manner, including those in regions where canola originated or is cultivated in significant quantities. We have participated in numerous conferences intended to enhance international cooperation on safety in biotechnology, and sponsored several workshops on safeguards for planned introductions of transgenic crops (crucifers, maize, wheat, potatoes, rice, tomatoes) most of which have included consideration of international biosafety issues.

In the course of these studies and interactions, APHIS has not identified any significant impacts on the environment that might be relevant to MS8 and RF3 canola or follow

from their unconfined cultivation in the United States and its territories, or abroad which could not be mitigated by reasonable agricultural practices. All the existing national and international regulatory authorities and phytosanitary regimes that currently apply to introductions of new canola cultivars internationally apply equally to those covered by the proposed determination.

V. CONCLUSIONS

In accordance with the requirements of NEPA, APHIS has considered the potential for significant impact on the environment of the proposed action and has reached the following conclusions:

1. The introduced genes, and their products, and the added regulatory sequences controlling their expression do not confer upon MS8 and RF3 canola or their progeny any disease or plant pest characteristic.

2. MS8 and RF3 canola and their progeny do not exhibit increased weediness potential relative to other commerical canola. Furthermore, introgression of their transgenes into canola or its sexually compatible relatives should not increase their weediness or impact biodiversity any more than gene introgression from commercial canola cultivars.

3. The use of MS8 and RF3 canola or their progeny in agriculture will not cause damage to raw or processed agricultural commodities.

4. The use of MS8 and RF3 canola or their progeny in agriculture will not have a significant impact on any beneficial organisms in the environment, or on any threatened or endangered species.

5. The use of MS8 and RF3 canola or their progeny in agriculture is unlikely to have any significant adverse impact on agricultural practices.

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APPENDIX A

RESPONSE TO AGREVO PETITION 98-278-01p FOR DETERMINATION OF NONREGULATED STATUS FOR CANOLA TRANSFORMATION EVENTS MS8 AND RF3 GENETICALLY ENGINEERED FOR POLLINATION CONTROL AND GLUFOSINATE HERBICIDE TOLERANCE

Prepared by
United States Department of Agriculture
Animal and Plant Health Inspection Service
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I. SUMMARY

The Animal and Plant Health Inspection Service (APHIS) has determined, based on a review of scientific data and information that canola (*Brassica napus* L.) transformation events MS8 and RF3 do not present a plant pest risk, and are therefore no longer considered regulated articles under 7 CFR Part 340. As a result of this determination, approval under those regulations will no longer be required from APHIS for planting or other environmental release, importation, or interstate movement within the United States and its territories of MS8 or RF3 canola or progeny derived from either of these transformation events. Exportation of this canola, and nursery stock or seeds capable of propagation will remain regulated according to the Foreign Quarantine Notices regulations at 7 CFR Part 319.

This determination by APHIS has been made in response to a petition (98-278-01p) received from AgrEvo USA Company (AgrEvo) on October 5, 1998 which requests a determination from APHIS that canola transformation events MS8 and RF3 should no longer be considered regulated articles because they do not present a plant pest risk. On December 8, 1998, APHIS announced receipt of this petition in the *Federal Register* (63 FR 67643-67644) and stated that the petition was available for public review. APHIS invited written comments on whether these canola transformation events pose a plant pest risk, to be submitted on or before February 8, 1999. No comments were received.

The subject canola transformation events were genetically engineered for male sterility (MS8), restoration of male fertility (RF3), and tolerance to the herbicide glufosinate (both MS8 and RF3), to enable the production of pure hybrid canola varieties by the use of a pollination control system. Two foreign genes controlling pollination, *barnase* and *barstar*, were stably integrated into the genome of canola variety Drakkar to produce transformation events MS8 and RF3, respectively. The *barnase* gene expresses a ribonuclease that blocks pollen development and results in male sterility in MS8 canola or progeny containing the gene. The *barstar* gene encodes a specific inhibitor of this ribonuclease which restores male fertility in plants containing the *barnase* gene. Thus, male fertile canola plants such as RF3 that express the *barstar* gene can be used in control pollinations of male sterile canola plants such as MS8 that contain the *barnase* gene to produce hybrid progeny with restored male fertility. The *barnase* and *barstar* genes were derived from the bacterium *Bacillus amyloliquefaciens*, and are linked in MS8 and RF3 to an inserted *bar* gene derived from the bacterium *Streptomyces hygroscopicus*. The *bar* gene encodes the enzyme phosphinothricin-N-acetyltransferase (PAT) that confers tolerance to the herbicide glufosinate. This trait allows for selection of plants during breeding that carry the linked pollination control genes and provides tolerance to glufosinate herbicides which could be used to control weeds during cultivation of MS8, RF3 or their progeny, provided the herbicide is registered for that purpose. The foreign genes were introduced into canola via an *Agrobacterium-*

mediated transformation procedure that has been widely used for over a decade for introducing various genes of interest directly into plant genomes.

APHIS regulations at 7 CFR Part 340 regulate the introduction (importation, interstate movement, or release into the environment, or any attempt thereat) of certain genetically engineered organisms and products. An organism is no longer subject to the regulatory requirements of 7 CFR Part 340 when it is demonstrated not to present a plant pest risk. Section 340.6 of the regulations, entitled "Petition for determination of nonregulated status", provides that a person may petition the Agency to evaluate submitted data and determine whether a particular regulated article does not present a plant pest risk, and therefore should no longer be regulated. If the agency makes such a determination and the petition is granted, then introduction of the regulated article could proceed without permits or notifications under 7 CFR Part 340.

MS8 and RF3 canola have been considered "regulated articles" because the plant pathogen *Agrobacterium tumefaciens* was used as a vector agent and as a source of noncoding sequences used to regulate the expression of inserted genes. As such, field trials of MS8 and RF3 canola and their progeny conducted in the U.S. were performed under conditions of physical and reproductive confinement as authorized by APHIS permits or notifications. Field tests have also been completed in Canada and Europe.

APHIS' determination that MS8 and RF3 canola transformation events will no longer be considered regulated articles under APHIS regulations at 7 CFR Part 340, is based on an analysis of field test data and other data provided by AgrEvo as well as other scientific information relating to their potential plant pest risk. From our review, we determined that MS8 and RF3 canola: (1) exhibit no plant pathogenic properties; (2) are no more likely to become weeds, or increase the weediness potential or effect biodiversity of sexually compatible relatives, any more than commercially available canola varieties; (3) will not cause damage to raw or processed agricultural commodities; (4) will not harm organisms beneficial to plants (e.g., bees and earthworms), or threatened or endangered species; and (5) are unlikely to have any significant adverse impact on agricultural practices. APHIS has also concluded that there is a reasonable certainty that new progeny varieties bred from MS8 and/or RF3 will not exhibit new plant pest properties, i.e., properties substantially different from any observed for MS8 or RF3 canola, or those observed for traditionally bred canola.

An Environmental Assessment (EA) has been prepared by APHIS for this determination in accordance with regulations and procedures implementing the National Environmental Policy Act (NEPA), as amended (42 U.S.C. 4321 *et seq.*); 40 CFR Parts 1500-1508; 7 CFR Part 1b; 7 CFR Part 372. The EA and the Finding of No Significant Impact (FONSI) reached are available from APHIS upon written request.

II. BACKGROUND

USDA Regulatory Authority. APHIS regulations, which were promulgated pursuant to authority granted by the Federal Plant Pest Act (FPPA), (7 U.S.C. 150aa-150jj) as amended, and the Plant Quarantine Act (PQA), (7 U.S.C. 151-164a, 166-167) as amended, regulate the introduction (importation, interstate movement, or release into the environment, or any attempt thereat) of certain genetically engineered organisms and products. A genetically engineered organism is deemed a regulated article if the donor organism, recipient organism, vector or vector agent used in engineering the organism belongs to one of the taxa listed in § 340.2 of the regulations and is also a plant pest; if it is unclassified; or if APHIS has reason to believe that the genetically engineered organism presents a plant pest risk. MS8 and RF3 canola have been considered "regulated articles" because the plant pathogen *Agrobacterium tumefaciens* was used as a vector agent and as a source of noncoding sequences used to regulate the expression of inserted genes.

Prior to the introduction of a regulated article, a person is required under § 340.1 of the regulations to either (1) notify APHIS in accordance with § 340.3 or (2) obtain a permit in accordance with § 340.4. Introduction under notification (§ 340.3) requires that specified eligibility criteria and performance standards are met. The eligibility criteria impose limitations on the types of genetic modifications that qualify for notification, and the performance standards impose limitations on how the introduction may be conducted. Under § 340.4, a permit is granted for a field trial when APHIS has determined that the conduct of the field trial, under the conditions specified by the applicant and/or stipulated by APHIS, does not pose a plant pest risk. MS8 and RF3 canola have been field tested in the U.S. since 1997 under APHIS permits and notifications. For certain genetically engineered organisms, field testing may be required to verify that they exhibit the expected biological properties, and to demonstrate that they do not pose plant pest risks as a result of the plant pest components or vectors used during the transformation or as a result of the transformation itself.

An organism is no longer subject to the regulatory requirements of 7 CFR Part 340 when it is demonstrated not to present a plant pest risk. Section 340.6 of the regulations, entitled "Petition process for determination of nonregulated status", provides that a person may petition the Agency to evaluate submitted data and determine that a particular regulated article does not present a plant pest risk and should no longer be regulated. If the agency determines that the regulated article does not present a risk of introduction or dissemination of a plant pest, the petition may be granted. A petition may be granted in whole or in part. MS8 and RF3 canola have been approved for cultivation, food and feed use in Canada. Following a plant pest risk assessment, on September 23, 1998 APHIS authorized importation from Canada into the U.S. of seed from MS8 and RF3 transformation events, or progeny derived from crosses between them or with other canola not subject to APHIS regulations at 7 CFR

Part 340, only for the express purpose of processing. The current petition from AgrEvo, if granted in whole, would release MS8 and RF3 canola from all regulatory requirements under 7 CFR Part 340 for all types of introductions.

APHIS believes it prudent to provide assurance prior to commercialization that organisms developed using biological vectors from pathogenic sources, transforming material from pathogenic sources, or pathogens as vector agents, have been evaluated to assure that there is not a plant pest risk. Such assurance may aid the entry of new plant varieties into commerce or into breeding and development programs. APHIS' determination of plant pest risk is based, in part, on any field data and other information either provided by the petitioner or available in the scientific literature concerning the biological properties of the regulated plant, and its similarity to other varieties of the same plant grown using standard agricultural practices for commercial sale or private use. A certification that an organism does not present a plant pest risk means that there is reasonable certainty that the organism cannot directly or indirectly cause disease, injury, or damage to plants, or organisms beneficial to plants, either when grown in the field, or when stored, sold, or processed. This approach is considerably broader than a narrow definition of plant pest risk arising from microbial or animal pathogens, including insect pests. Other traits, such as increased weediness, and harmful effects on beneficial organisms, such as earthworms and bees, are clearly subsumed within what is meant by direct or indirect plant pest risk.

EPA and FDA regulatory authority. MS8 and RF3 canola are currently subject to regulations administered by the EPA or the FDA regarding food and feed safety as described in the Environmental Assessment. FDA granted a finding of 'No Concern' for canola transformation events MS8 and RF3 in September 1998 following its consultation with AgrEvo on food and feed safety for these transgenic canola. Full registration and tolerance establishment for use of glufosinate-ammonium herbicide Liberty® on glufosinate-tolerant canola (such as MS8 and RF3 canola) is pending with the EPA.

III. RATIONALE FOR DEVELOPING MS8 AND RF3 CANOLA

According to the petitioner, producing higher yielding oilseed rape varieties is a major goal of oilseed rape breeders. This is most effectively accomplished by the use of F1 hybrids, which are estimated to yield 20-25% more seeds and are more uniform than the best open-pollinated varieties. Oilseed rape is capable of both self-pollination (70%) and cross-pollination (30%), thus control of pollination is required to produce 100% F1 hybrid seeds. The subject canola transformation events were genetically engineered to express genes for male sterility (MS8), restoration of male fertility (RF3), and tolerance to the herbicide glufosinate (both MS8 and RF3), to enable the production of pure hybrid canola varieties by the use of a new type of pollination control system. Male

fertile RF3 canola plants can be used in control pollinations of male sterile MS8 canola plants to produce pure hybrid progeny with restored male fertility. The pollination control traits in MS8 and RF3 are linked to the glufosinate herbicide tolerance trait. This trait allows for selection of plants during breeding that carry the linked pollination control genes and provides tolerance to glufosinate herbicides which could be used to control weeds.

Weed management is critical to maximize crop yield and obtain high-quality seed harvest free of weed seeds; but it is an expensive and labor intensive operation. Glufosinate-tolerant canola offers farmers an additional option for post-emergent weed control. Often farmers use pre-emergent herbicides that will stop weeds seeds from germinating. However, this assumes that weeds will always be a problem in all parts of the field. With glufosinate-tolerant canola, farmers will have the option of applying appropriately registered glufosinate-containing herbicide to control weeds after they have germinated and only in the areas of the field where there are weeds. Applications in this manner may reduce the amount of pre-emergent herbicide used on canola. Glufosinate may also control a broader range of weeds in canola than other individual, currently registered herbicides.

IV. ANALYSIS OF THE PROPERTIES AND PLANT PEST RISK POTENTIAL OF MS8 AND RF3 CANOLA AND THEIR PROGENY

A brief description of the biology, taxonomy, cultivation, and seed production practices of canola is expected to be helpful in specific environmental and biosafety issues applicable to MS8 and RF3 canola. In addition, to reach its determination that MS8 and RF3 canola do not present a plant pest risk, APHIS has also analyzed data presented by AgrEvo in this petition and in a previous petition (97-205-01p) for determination of nonregulated status for glufosinate-tolerant canola transformation event T45, and scientific data on other topics relevant to a discussion of plant pest risk. Based on this analysis, APHIS has arrived at a series of conclusions regarding the properties of MS8 and RF3 canola and progeny derived from these transformation events.

Biology and cultivation of canola. *Brassica napus* L., is a mustard crop grown primarily for its seed which yields about forty percent oil and a high-protein animal feed. Varieties of *B. napus* are known by the common names of rapeseed, rape, oilseed rape, and canola. Major canola producing states in the U.S. are North Dakota, Minnesota, Montana, Idaho, Washington, and Georgia. The maturity group 00 oilseed rape variety Drakkar was the parental variety used for transformation. This variety is common spring variety in the canola growing regions of western Canada and Europe.

Taxonomy of rapeseed. *Brassica* is a genus within the plant family Brassicaceae (Cruciferae), which is commonly known as the mustard family. This family of about

375 genera and 3,200 species includes species recognized as crops, condiments, ornamentals, and many weeds. *Brassica* contains about 100 species, including cabbage, cauliflower, broccoli, brussels sprouts, turnip, various mustards and weeds (Willis 1973). *B. napus* belongs to a group of six genetically related species with different genome compositions and ploidy levels (Röbbelen et al. 1989):

B. nigra (L.) Koch, black mustard, a diploid species n=8 (bb genome), originally spread by trade over much of the Old World, and now spread as a weed throughout much of the New World, including virtually all of the United States.

B. oleracea L., cabbage, broccoli, brussels sprouts, cauliflower, kale, a diploid species n=9 (cc genome), originally confined to the Mediterranean, but now widely grown in temperate gardens.

B. rapa L. (=*B. campestris* L.), field mustard, turnip, turnip rape, bird rape, a diploid species n=10 (aa genome), originally spread throughout much of Europe, Asia, northern India, and northern Africa, and now either grown as a vegetable or oil crop, or spread as an occasional weed in much of the United States.

B. carinata A. Braun, Abyssinian mustard, Ethiopian mustard, an allotetraploid species n=17 (bb cc genomes), derived from *B. nigra* and *B. oleracea*, presumed to come from an ancient cross or crosses in northeast Africa, and occasionally grown in the United States as a novelty.

B. juncea (L.) Czerniakowska et Cosson, Indian mustard, brown mustard, mustard greens, an allotetraploid species n=18 (aa bb genomes), derived from Old World crosses of *B. nigra* and *B. rapa*, and now grown for the leaves, or spread as an occasional weed in crops or waste places.

B. napus L., the subject of this petition, an allotetraploid species n=19 (aa cc genomes), derived from ancient crosses between *B. oleracea* and *B. rapa*, and now grown widely for its oil, and an occasional weed or volunteer in cultivated fields.

Sexual reproduction and inter-specific crosses in rapeseed. *B. napus* produces an inflorescence of yellow, nectar-bearing, entomophilous flowers. The plants are capable of both self-fertilization and intra-specific cross-fertilization. Partial sexual compatibility also exists with some related *Brassica* spp. and other closely related species outside the genus.

In cultivated fields, cross-pollination in rapeseed has been reported at about 35%, but varies depending on the availability of insect pollinators, cultivar, and weather. Downey and Bing (1990) reported outcrossing rates of 2.1, 1.1, and 0.6 percent for isolation

plots located 46, 137, and 366 meters from a pollen source. Seed certification requires a reproductive isolation distance of 660 feet for the production of Foundation Seed for *B. napus*, and even greater distance (1320 feet) for self-incompatible species such as *B. rapa*. At these distances there is a tolerance of 0.05 percent off types, presumably derived from pollen contamination by sources beyond the specified distance (7 CFR Part 201.76). Care is taken to isolate a seed production field from contaminating weeds. Cytoplasmic male sterility is currently used to produce hybrid canola seed. However, the *pol* cytoplasm, the most common male-sterility inducing cytoplasm used throughout the world, is subject to high temperature reversion, and 100% hybrid seed is difficult to obtain (Pinnisch and McVetty, 1994).

Honey bees are the primary pollinators of rapeseed. Although a honeybee colony may collect nectar and pollen from many species, and potential foraging flights can be quite distant (to 10 km), several factors limit the potential for spread (Seeley, 1985). First, each individual honeybee forager almost always collects nectar and pollen from a single plant species during a single visit. Second, given abundant flowers, such as in a cultivated field, individual honeybee foragers tend to collect nectar and pollen from flowers in the same or immediately adjacent plants. Third, honeybees are very sensitive to barometric pressure, and decrease foraging distances in response to impending adverse weather. Fourth, honeybees generally do not forage at great distances from the nest when abundant nectar and pollen sources are close by, as in many agricultural settings.

Whereas intra-specific crosses between *B. napus* cultivars occur readily, inter-specific crosses between *B. napus* and related species occur with varying degrees of success and are influenced greatly by the direction of the cross. The three allotetraploid species mentioned above (*B. napus*, *B. juncea*, and *B. carinata*) undoubtedly arose from ancient natural crosses of diploid species, and therefore demonstrate the potential for gene movement among all these species. When *B. napus* is used as the female parent and when the species have at least one genome in common, the interspecific crosses are more successful (Renard et al. 1993; OECD, 1997; Scheffler and Dale, 1994) The potential for gene introgression from *B. napus* into its sexually compatible relatives is discussed in more detail below.

Neither the introduced genes, their products, nor the added regulatory sequences controlling their expression presents a plant pest risk.

A disarmed *Agrobacterium tumefaciens* system was used to transfer the new genetic material into the parental Drakkar variety to produce canola transformation events MS8 and RF3 (De Block et al., 1989). This transformation system is well documented to transfer and stably integrate T-DNA containing genes of interest into a plant nuclear chromosome (White, 1989, Howard et al., 1990). Although the transformation process uses the plant pathogen, *A. tumefaciens* (the causal agent of a tumor-inducing, crown

gall disease), the genes that cause crown gall disease are removed from the tumor-inducing (Ti)- plasmid, and therefore the transformed plant does not develop crown gall disease.

Sequences necessary for the expression of the desired trait were introduced between the left and right T-DNA borders from a disarmed Ti-plasmid (pTiB6S3) to create the chimeric plasmid vectors pTHW107 and pTHW118. AgrEvo data demonstrated for MS8 plants that a single copy of the T-DNA inserted into the plant genome at a single locus; and for RF3 plants, that one complete T-DNA copy arranged in an inverted repeat structure with a second, incomplete T-DNA copy inserted into the plant genome at a single locus. As expected, the data also demonstrate that the integrated DNA is restricted to the DNA comprised between the T-DNA border in the plasmid vectors described above. Sequences from the plasmid vectors outside of the T-DNA border repeats, including bacterial origins of replication (pBR *ori* and pVS1 *ori)* and a bacterial marker gene that confers streptomycin resistance and a bacterial *barstar* gene encoding a ribonuclease inhibitor, are **not** present in MS8 and RF3 transformants.

Genes and noncoding sequences necessary for their expression that are contained in the T-DNA inserted into MS8 and RF3 transformation events are as follows (full references for these sequences can be found in the petition).

The following sequences are responsible for the glufosinate herbicide tolerance trait in both MS8 and RF3. The coding sequence of the antibiotic bialaphos resistance gene (*bar*) of *Streptomyces hygroscopicus* (Thompson et al., 1987), of which the two N-terminal codons have been modified to ATG and GAC, encodes the enzyme phosphinothricin-N-acetyl transferase (PAT). PAT causes acetylation of the herbicide glufosinate-ammonium (a synthetic derivative of bialaphos) thereby rendering it inactive. The promoter from the S1A ribulose-1,5-bisphosphate carboxylase small subunit gene from the plant *Arabidopsis thaliana* (PSsuAra) (Krebbers et al., 1988), drives expression of the *bar* gene in green plant tissues. RF3 canola contains an additional incomplete copy of a non-functional part of this promoter. The 3' untranslated end from the T-DNA gene 7 (3'g7) of pTiB6S3 from *A. tumefaciens* provides sequences necessary for polyadenylation of mRNA for the inserted *bar* gene.

MS8 and RF3 contain in addition, the following sequences necessary for pollination control. MS8 contains the coding region of the *barnase* gene from *Bacillus amyloliquefaciens* (Hartley, 1988) and the 3'untranslated region downstream from this gene. The *barnase* gene encodes a specific ribonuclease enzyme which when expressed in the tapetal cell layer of anthers, blocks pollen development and results in male sterility (Hartley, 1989; Mariani et al., 1990; De Block et al., 1992). RF3 contains two complete copies of the coding region of the *barstar* gene, also derived from *B. amyloliquefaciens* (Hartley, 1988), and the 3'untranslated region downstream from this gene. The *barstar* gene encodes a specific protein inhibitor of the Barnase ribonuclease

protein (Hartley, 1989). Co-expression of both *barnase* and *barstar* in anthers prevents male sterility caused by the *barnase* gene (Mariani et al., 1992). Anther-specific expression of both the *barnase* and *barstar* gene are controlled by the promoter region of the TA29 gene from tobacco (*Nicotiana tabacum*) (Seurinck et al., 1990). Sequences necessary for polyadenylation of mRNA for the inserted *barnase* and *barstar* genes are provided by the 3' untranslated sequence from the nopaline synthase gene (3'*nos*) from *A. tumefaciens*. The second copy of the *barstar* gene in RF3 resulting from the insertion of the incomplete copy of a second T-DNA, as described above, is under the control of a truncated, but functional, part of the TA29 promoter and a second complete copy of 3' *nos*.

AgrEvo inheritance data demonstrate: (1) that glufosinate tolerance conferred by the *bar* gene (linked to *barnase* and *barstar* in MS8 and RF3, respectively) is inherited in a stable Mendelian manner as a single dominant locus over at least 2 backcross generations in different genetic backgrounds of spring oilseed rape (Section V.b. pg. 34, Table 5, and Amendment 1 of the petition, Table 6) ; (2) that the male sterility trait in MS8 also is inherited in a stable Mendelian manner as a single dominant locus; and (3) that RF3 plants homozygous for the *barstar* gene are capable of restoring male fertility 100% in progeny from crosses of a male sterile line containing the *barnase* gene (Amendment 1, Attachment 4 of the petition).

Although 3' untranslated DNA sequences from both the nopaline synthase gene and gene 7 from the plant pest *Agrobacterium* were inserted into MS8 and RF3 canola, these sequences cannot incite disease. Furthermore, initial transformed tissue was treated with an appropriate antibiotic (e.g. carbenicillin) to eliminate the *Agrobacterium* (De Block et al., 1989); and no crown gall symptoms were reported in these canola by AgrEvo under field conditions.

Furthermore, AgrEvo provides evidence that expression of the introduced genes does not result in disease symptoms or the synthesis of products toxic to other organisms. AgrEvo monitored field trials conducted with these transformation events or their hybrid progeny in 2 locations in North Dakota and Wisconsin in 1997 and at 14 locations in Minnesota, North Dakota, Wisconsin, and Idaho in 1998 to evaluate agronomic characteristics and performance of the hybrid MS8/RF3 compared to the nontransgenic parent (Drakkar). The hybrids exhibited similar agronomic behavior as Drakkar regarding seed germination rates, plant stand, plant vigor, flowering times, deleterious effects, and disease and pest resistance or susceptibility (Petition: pg. 41, and Field Data reports - Appendix 5). These observations are supported by the results of field trials conducted with MS8, RF3 and their hybrid combination during 1994 and 1995 in Canada (Saskatchewan) and in 1995 in Belgium. A variety of insect pests (e.g. aphids, cabbageworms, flea beetles, diamondback moth larvae, Bertha armyworm (*Mamestra configurata*), blister beetles, and pollen beetles), pollinators (honey bees and

bumble bees), and various flies, wasps, and mosquitoes were observed in one or more of these field trials in both the transgenic and nontransgenic canola.

MS8 and RF3 canola are not weeds; and introgression of the transgenes into canola or its sexually compatible relatives should not increase their weediness or impact biodiversity any more than gene introgression from commercial canola cultivars.

Weediness can be broadly defined as any capacity for unwanted invasion of natural habitats. Despite its ability to volunteer, escape from cultivated fields, and form temporary occasional populations, the parent plant in this petition, *Brassica napus*, is not a serious weed under conditions found in the United States. Although *B. napus* is listed as a common weed in small grains in New Jersey, it is not specifically listed as a troublesome weed in the U.S. in those crops surveyed by the Weed Science Society of America (1992), even in major canola producing states. The comprehensive world list of Holm et al. (1991) does not list it as a serious or principal weed anywhere in the world; they do, however, give two listings as a common weed: one in Finland and one in Kenya. *B. napus* is mentioned as an "occasional weed" by Munz (1968), and "sometimes escaped" by Bailey (1949). Generally most crop plants are bred and carefully selected to express agriculturally useful traits, and therefore, they are not usually competitive in unmanaged or untended natural environments. Without favorable conditions, and intensive cultivation, domesticated types of *B. napus* cannot compete successfully with naturalized forms of *B. napus* in the United States. Naturalized types of *B. napus* are sporadically distributed in Canadian environments, whereas in the United Kingdom, they are widespread in the wild, although they have not been classified as weeds (Mitchell-Olds, 1992; Holm et al., 1991). Efforts are under way to confirm whether these widespread canola are self sustaining populations or are a result of repeated introductions (van der Meijden and de Vries, 1992).

AgrEvo has submitted evidence to indicate that MS8 and RF3 canola or their hybrid are no more weedy than nontransgenic canola cultivars under agricultural conditions. Field observations indicate that seed germination and dormancy, seed production, pest and disease resistance characteristics, time to flowering, and sensitivity to herbicides other than glufosinate-ammonium are similar for MS8/RF3 hybrids and nontransgenic canola.

There is no reason to believe that the new traits engineered into MS8 and RF3 canola would by themselves, cause these canola to be more weedy. These genetic alterations do not result in characteristics commonly observed in many of the world's worst weeds (Baker, 1965). Another glufosinate-ammonium tolerant canola transformation event (T45) deregulated by APHIS exhibited no increased weediness potential (USDA, 1998). As previously noted, glufosinate tolerance, is unlikely to increase weediness of canola unless glufosinate is the only alternative for control of the plant. Such an alteration, because it does not confer any pest resistance or alter reproductive biology or change

any physiology related to survival, does not confer a competitive advantage favoring the canola plants over unmodified varieties. To increase weediness of the canola plant there would have to be selection pressure on glufosinate tolerant canola (Tiedje et al., 1989; Office of Technology Assessment, 1988). Moreover, AgrEvo presents evidence that MS8 and RF3 canola are still susceptible to other herbicides that control mustards (e.g. glyphosate, phenoxys, and sulfonylureas).

Transgenic, glufosinate-tolerant canola have been tested for increased invasiveness under field conditions in the United Kingdom (Cherfas, 1991, Crawley, 1992; Crawley et al. 1993). The major conclusions of these studies are that transgenic canola is not any more aggressive than the nontransgenic canola, transgenic rapeseed do not invade undisturbed habitats, and they do not persist in the environment into which they were introduced any more than their parents did. In addition, after two years of monitoring the occurrence and fate of glufosinate tolerant canola volunteers and weedy relatives following the growth of glufosinate-tolerant varieties in the 1995 growing season in Saskatchewan, AgrEvo Canada concluded that:

(1) glufosinate tolerant canola behaves no differently as a volunteer than does standard non-transgenic canola,

(2) outcrossing did not result in the transfer of glufosinate tolerance to weedy relatives

(3) familiar management practices (e.g. crop rotation, the use of alternative herbicides, mowing of ditches and roadsides) are the key to controlling volunteer canola (transgenic or otherwise) and its weedy relatives (AgrEvo Canada, 1998).

The male sterility and male fertility restoration traits engineered into MS8 and RF3 canola would not be expected to increase the weediness potential of canola. In fact, male sterility alone would provide a significant disadvantage to seed production and thus persistance of MS8 canola in natural habitats where canola pollen from other sources may be limiting. Male sterility in MS8 is unlikely to increase the weediness potential anymore so than would cytoplasmic-male sterility used for the production of hybrid spring oilseed rape cultivars. Fertility of RF3 plants was reported to be similar to the nontransformed parent, and these plants will not affect the male fertility of plants that lack the *barnase* gene. AgrEvo field trial data show no obvious change in characteristics that would lead to an increased weediness potential in MS8 or RF3 canola or their hybrids.

Introgression of the transgenes in MS8 and RF3 canola into sexually compatible relatives should not increase their weediness or impact biodiversity any more than gene introgression from commercial canola cultivars. Table 1. in the petition summarizes data compiled from differences sources on the potential of *B. napus* to form hybrids with related Brassicaceae species in the U.S. when used as the pollen donor under field conditions, and the fertility of hybrids produced. No hybrids were reported with *B. oleracea*, *B. carinata*, *B. elongata*, or *B. tournefortii*, and these species are not found in

the major canola producing states. In addition, no hybrids were reported with the more distantly related *Sinapsis arvensis* syn. *B. kaber* (wild mustard) or *Sinapsis alba* syn. *B. hirta*, or *Diplotaxis muralis*. Of these latter species, the *Sinapsis* species occur in all of the major canola producing states.

Hybrids were most readily formed with *B. rapa* (rates ranging to 93%) and fertility of those hybrids ranged from < 10% to 86%, depending on the reference (Bing et al., 1991; Jørgensen and Anderson, 1994). Hybrids were reported at extremely low rates for *B. nigra* and *B. juncea*, but *B. nigra* hybrids were male sterile and fertility of *B. juncea* hybrids was extremely low.

Hybrids have also been made in field crosses using *B. adpressa*, syn. *Herschfeldia incana* (hoary mustard) (Lefol et al., 1995) and *Raphanus raphanistrum* (wild radish) (Baranger et al., 1995; Eber et al., 1994; Chévre et al., 1997) as pollen donors and male sterile oil seed rape (*B. napus*), containing the Ogura male sterile cytoplasm derived from wild radish, as the female parent. Lefol et al. (1995) conclude that hybrids with *B. adpressa* may be more vegetatively competitive than hoary mustard in cultivated or non-cultivated areas, but the weediness of these plants should not be a cause of concern in cultivated fields. Due to varying degrees of infertility in the F1, reproductive capacity was not evaluated. Introgression into hoary mustard is unlikely due to chromosome incompatibilities (Eber et al., 1994). Crosses with *R. raphanistrum* resulted in the production of a low percentage of hybrids which were triploid and had low fertility. Triploidy would make further crosses back to either parent difficult; however, introgression is possible when *R. raphanistrum* exists at artificially high densities compared to male-sterile *B. napus* (Chévre et al., 1997).

All of these species (*B. rapa*, *B. nigra*, *B. juncea*, *B. adpressa*, and *R. raphanistrum*) are found in the major canola producing states. Thus the potential would exist for transgene introgression from MS8 or RF3 or its hybrid to occur at a relatively low to moderate rate into *B. rapa*, and at extremely low rates for *B. juncea*, *B. adpressa*, *B. nigra*, and *R. raphanistrum*.

Reduced dormancy of *B. rapa* x *B. napus* hybrids relative to the persistent wild *B. rapa*, coupled with the reduced fertility of the inter-specific hybrid makes it very unlikely that populations of these hybrids will persist. There is a small chance that hybrids could backcross to wild *B. rapa* and thereby transfer the transgenes to wild populations (Crawley et al., 1993).

Many species of *Brassica* and related mustards are weeds or have weedy tendencies. *B. juncea*, *B. nigra*, *B. rapa*, and *S. arvensis* (=*B. kaber*) to some degree are agricultural weeds, sometimes serious, in much of the United States (Gleason, 1952; Slife et al., 1960; Reed, 1970; Muenscher, 1980). In Europe, *B. rapa* is a common weed in

agricultural fields, and introgression of an herbicide resistance transgene from *B. napus* canola to wild *B. rapa* has been detected (Mikkelsen et al., 1996).

Since MS8 and RF3 canola and their hybrids do not exhibit weedy characteristics or have any fitness advantage as a result of the transgenes, and due to the lack of selection pressure for these expressed traits outside of cultivation, transgene introgression into the sexually compatible relatives described above is unlikely to increase their weediness or impact their biodiversity anymore than would gene introgression from other canola cultivars currently available, including other nontransgenic, herbicide tolerant or cytoplasmic male sterile canola cultivars. Introgression of the *barnase* transgene in the absence of the *barstar* gene would most likely result in male sterility. Since these two genes are not linked, independent segregation would be expected. In agricultural settings, introgression of the glufosinate tolerant transgene into weedy relatives may provide a competitive advantage if glufosinate is used for weed management, however, other herbicides or mechanical means can be used to control such weeds.

There is no published evidence for the existence of any mechanism, other than sexual crossing of compatible Brassicaceae species, by which the introduced genetic sequences can be transferred to other organisms. Another mechanism by which *B. napus* can transfer genetic material to sexually non-compatible plants is through "bridging". Bridging occurs when a mating is made between two incompatible or reproductively isolated species by first transferring the genetic material to an intermediate species that is sexually compatible with the two sexually incompatible species. Such a possibility of the "bridging" phenomenon may occur with *B. juncea* acting as the intermediate species. The occurrence of hybrids between *B. napus* and *B. juncea* is rare, and moreover, the hybrids do not persist long enough in the environment due to poor fertility, poor germination, and high seedling mortality, to serve as a bridge species. Another barrier for gene transfer is that chromosomal crossing over in the *B. napus* and *B. juncea* hybrid must occur for stable gene introduction into *B. nigra* (Scheffler and Dale, 1994).

Comparative analyses of numerous gene sequences from microorganisms and plants have never, to our knowledge, yielded any published evidence of strong inter-kingdom gene homologies that would be indicative of recent or frequent gene exchanges between plants and microorganisms with the exception of T-DNA of the Ti-plasmid of *Agrobacterium*. There is some scientific literature (e.g., Carlson and Chelm, 1986; Wakabayashi et al., 1986) that provides a suggestion that transfer of genes from plants to microorganisms may have occurred over evolutionary time, i.e., in the eons since the various times of divergence between the kingdoms. Bryngelsson et al. (1988) have suggested that plant DNA can be taken up by a parasitic fungus, but no evidence has ever been forthcoming that such DNA uptake has resulted in the frequent transfer of a functional DNA sequence. Even if a rare plant-to-microbe gene transfer were to occur, there is no reason to believe that such a transfer of any of the sequences would pose any plant pest risk. Any concerns regarding transfer of the new genetic material inserted

into MS8 and RF3 canola into microorganisms are, at best, highly speculative, and improbable, if not altogether impossible.

MS8 and RF3 canola will not cause damage to agricultural commodities.

The FDA has issued a finding of 'No Concern' to AgrEvo for these canola transformation events in September 1998, and the use of these canola for food and feed purposes has also been granted by Canada. The proteins Barnase ribonuclease, Barstar ribonuclease inhibitor, and PAT do not pose any safety concern. AgrEvo data demonstrate that, as expected, the genes encoding these proteins are not expressed (or are expressed at extremely low levels) in the seed, because these genes are under the control of tissue-specific promoters that express only in the anthers (*barnase* and *barstar*) and green tissue (*bar*) (Petition, Fig. 9. and Tables 7-9).

Canola, by definition, is specifically bred to have extremely low levels of toxicants, although *B. napus* rapeseed and its close relatives are known to carry several toxicants (Bell, 1984; Busch et al. 1994; Cheeke, 1989). Erucic acid and glucosinolates are the only two toxicants known in rapeseed. Erucic acid is a monounsaturated fatty acid (22:1) normally produced in very high concentrations (20-60%) in rapeseed. Canola, by definition has less than 2% of erucic acid which is considered safe. Field production of crops that produce high levels of erucic acid for industrial purposes is not restricted or otherwise regulated in the United States. Canola varieties also have very low levels (the range of about 6 to 16 micromole/g) of alkyl glucosinolates in the defatted seed meal. MS8 and RF3 canola has been developed from low erucic acid and low glucosinolate canola varieties, and these transformation events were selected, in part, for normal oil and seed quality. AgrEvo confirmed that the erucid acid level was not higher than that expected (0.05% of the total oil composition) for a double zero variety such as Drakkar. As such, MS8 and RF3 canola should not present any concerns as far as toxicological properties of canola.

APHIS notes that Agriculture and Agri-Food Canada (1996) concludes that AgrEvo demonstrated that the nutritional composition of the whole seed, processed meal or oil derived from MS8, RF3, and their hybrid is substantially equivalent to conventional canola varieties. APHIS concludes that MS8, RF3 and their hybrid should not have a direct or indirect plant pest effect on any processed commodity.

MS8 and RF3 canola will not be harmful beneficial organisms, including bees, or to endangered or threatened species.

There is no reason to believe that deleterious effects on beneficial organisms could result from the cultivation of MS8 or RF3 canola or their hybrid. First the traits controlling pollination are expected to be expressed only in the tapetum of the anthers. Expression data and phenotypic observations of these plants support this conclusion.

AgrEvo reports that the male sterility trait conferred by the *barnase* gene has minimal effects on flower morphology. Although pollen is not produced, flower nectaries, which provide a source of nutrients for pollinators, develop normally, and the flowers do not show a greater tendency towards bud abortion (Petition pg. 43). The RF3 plants and the hybrids have normal flower morphology, fertility, and attractiveness to insect pollinators. Normal insect activity was observed on all these plants. The new transgene proteins expressed in the transgenic canola plants were derived from common soil bacteria, and ribonucleases and ribonuclease inhibitors are common in bacteria and plants. Therefore these proteins or similar proteins are normal parts of the diets of animals, humans and insects. Cabbage seedpod weevil (*Ceutorhynchis assimilis*) and other *Lygus* species are common pests of canola. These insects are not on the list of threatened and endangered species. Other glufosinate tolerant canola transformation events have not been shown to be harmful to beneficial organisms or threatened and endangered species (USDA, 1998). MS8 and RF3 canola and their hybrid do not contain elevated level of toxic oils, and therefore, insects that may feed on these canola will not be unduly affected in their ability to reproduce or function normally after feeding. Knowledge of the mode of action, and the lack of known toxicity for the newly expressed proteins suggest no potential for deleterious effects on beneficial organisms such as bees and earthworms. Results of trials in the United States, Canada, and Europe do not reveal any noticeable adverse effects on beneficial organisms. APHIS has identified no other potential mechanisms for deleterious effects on beneficial organisms following from the cultivation of MS8 and RF3 canola.

MS8 and RF3 canola will not have a negative impact on agricultural and cultivation practices.

Based on APHIS' analysis, there is unlikely to be any significant adverse impact on agricultural practices associated with the use of these canola. Canola seed can remain in the soil profile and produce volunteer plants that may be considered weeds in subsequent crop rotations. If glufosinate-tolerant canola volunteers occur in rotations with other glufosinate-tolerant crops currently on the market (such as soybeans or corn) or on uncultivated land, glufosinate could not be used to manage them as weeds. Glufosinate-tolerant canola has been in commercial production in Canada since 1996, and AgrEvo notes that control of glufosinate-tolerant canola volunteers can be achieved through the use of broadleaf herbicides like glyphosate, 2,4-D and sulfonylurea type herbicides, depending on the crop. They note that normal crop and herbicide rotations have been effective in controlling such volunteers in commercial production (AgrEvo Canada, 1998). Because other canola varieties tolerant to herbicides with different modes of action (e.g. glyphosate) may be commercially available in the U.S. (as well as Canada), APHIS is aware of the concern that there is a likelihood of canola volunteers possessing a combination of two different herbicide resistance genes via crossing and how such volunteers would be managed by growers. Mechanical means or appropriate alternative herbicides with different modes of action available for each of the major

crops in a typical rotation could be used to manage such volunteers (USDA, 1999; Monsanto Company, 1998, Petition 98-216-01p, see Table 9). The Canadian Government has outlined the need for sound crop management practices for volunteer management in its Document DD96-17 (Agriculture and Agri-Food Canada, 1996).

V. CONCLUSIONS

APHIS has determined that MS8 and RF3 canola transformation events will no longer be considered regulated articles under APHIS regulations at 7 CFR Part 340. Permits or notifications under those regulations will no longer be required from APHIS for field testing, importation, or interstate movement of these canola or progeny derived from these transformation events. Importation of these canola, and nursery stock or seeds capable of propagation, is still, however, subject to the restrictions found in the Foreign Quarantine Notice regulations at 7 CFR Part 319. This determination has been made based on an analysis which revealed that the canola transformation events MS8 and RF3 and their hybrid progeny: (1) exhibit no plant pathogenic properties; (2) are no more likely to become weeds than the non-engineered parental variety, and are unlikely to increase the weediness potential for any other cultivated plant or native wild species with which these canola can interbreed; (3) will not cause damage to raw or processed agricultural commodities; (4) will not harm endangered or threatened species or other organisms, such as bees, that are beneficial to agriculture; and (5) are unlikely to have any significant adverse impact on agricultural practices. APHIS has also concluded that there is a reasonable certainty that new progeny varieties bred from these canola transformation events will not exhibit new plant pest properties, i.e., properties substantially different from any observed during their field testing, or those observed for canola in traditional breeding programs.

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