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Petition for the Determination of Nonregulated Status for Glyphosate-Tolerant Canola MON 88302

The undersigned submits this petition under 7 CFR § 340.6 to request that the Administrator make a determination that the article should not be regulated under 7 CFR Part 340

June 27, 2011

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RELEASE OF INFORMATION

Monsanto is submitting the information in this petition for review by the USDA as part of the regulatory process. By submitting this information, Monsanto does not authorize its release to any third party. In the event the USDA receives a Freedom of Information Act request, pursuant to 5 U.S.C., § 552, and 7 CFR Part 1, covering all or some of this information, Monsanto expects that, in advance of the release of the document(s), USDA will provide Monsanto with a copy of the material proposed to be released and the opportunity to object to the release of any information based on appropriate legal grounds, *e.g*., responsiveness, confidentiality, and/or competitive concerns. Monsanto understands that a copy of this information may be made available to the public in a reading room and upon individual request as part of a public comment period. Except in accordance with the foregoing, Monsanto does not authorize the release, publication or other distribution of this information (including website posting) without Monsanto's prior notice and consent.

CERTIFICATION

The undersigned certifies that, to the best knowledge and belief of the undersigned, this petition includes all information and views on which to base a determination, and that it includes all relevant data and information known to the petitioner that are unfavorable to the petition.

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EXECUTIVE SUMMARY

The Animal and Plant Health Inspection Service (APHIS) of the United States (U.S.) Department of Agriculture (USDA) has responsibility under the Plant Protection Act (Title IV Pub. L. 106-224, 114 Stat. 438, 7 U.S.C. § 7701-7772) to prevent the introduction and dissemination of plant pests into the U.S. APHIS regulation 7 CFR § 340.6 provides that an applicant may petition APHIS to evaluate submitted data to determine that a particular regulated article does not present a plant pest risk and no longer should be regulated. If APHIS determines that the regulated article does not present a plant pest risk, the petition is granted, thereby allowing unrestricted introduction of the article.

Monsanto Company is submitting this request to APHIS for a determination of nonregulated status for the new biotechnology-derived canola product, MON 88302, any progeny derived from crosses between MON 88302 and conventional canola, and any progeny derived from crosses of MON 88302 with biotechnology-derived canola that have previously been granted nonregulated status under 7 CFR Part 340.

Product Description

Weed competition can be a major limiting factor in canola production leading to significant yield reductions. Monsanto Company has developed a second-generation glyphosate-tolerant canola product, MON 88302, designed to provide growers with improved weed control through greater flexibility for glyphosate herbicide application. MON 88302 produces the same 5-enolpyruvylshikimate-3-phosphate synthase (CP4 EPSPS) protein that is produced in commercial Roundup Ready® crop products, via the incorporation of a *cp4 epsps* coding sequence. The CP4 EPSPS protein confers tolerance to the herbicide glyphosate, the active ingredient in the family of Roundup agricultural herbicides.

MON 88302 utilizes an improved promoter sequence to enhance CP4 EPSPS expression in male reproductive tissues (*i.e.*, pollen), compared to the promoter used to drive CP4 EPSPS production in the first-generation product, Roundup Ready canola (RT73). Enhanced CP4 EPSPS expression in the male reproductive tissues of MON 88302 allows the greater flexibility of glyphosate herbicide applications as MON 88302 plants can be sprayed with higher rates of glyphosate and at later stages of development with no detectable impact to male fertility. Glyphosate is a systemic herbicide and is translocated in the plant, generally from a strong source tissue (*e.g.,* leaf) to rapidly developing, or sink tissue. Sink tissues, such as pollen, that accumulate glyphosate and lack sufficient CP4 EPSPS expression are considered to be at risk for glyphosate injury. By virtue of enhanced CP4 EPSPS expression in male reproductive tissues, MON 88302 provides tolerance to glyphosate during the sensitive reproductive stages of growth, and enables the application of glyphosate at higher rates and at later stages of development than is possible with the current Roundup Ready canola product.

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Use of MON 88302 will enable growers to apply Roundup herbicide at higher rates and at later stages of development than is possible with the current product facilitating: 1) better control of tough-to-kill weeds; 2) an increased opportunity to control weeds if glyphosate application is delayed due to weather or equipment failure; 3) an enhanced ability to tailor labeled glyphosate applications to the weed development stage instead of the canola developmental stage; and 4) enhanced protection of canola plants at more advanced development stages. Use of MON 88302 will provide growers with the opportunity to ensure weeds that may impact yields are removed at the optimal time while minimizing the potential for crop injury.

Data and Information Presented Confirms the Lack of Plant Pest Potential and the Food and Feed Safety of MON 88302 Compared to Conventional Canola

The data and information presented in this petition demonstrate MON 88302 is agronomically, phenotypically, and compositionally comparable to conventional canola, with the exception of the introduced trait. Moreover, the data presented demonstrate MON 88302 is unlikely to pose an increased plant pest risk, including weediness or adverse environmental impact, compared to conventional canola. The food, feed and environmental safety of MON 88302 was confirmed based on multiple, well established lines of evidence:

- Canola is a familiar crop that has a history of safe consumption, and serves as an appropriate basis of comparison for MON 88302.

- A detailed molecular characterization of the introduced DNA demonstrated a single, intact copy of the transgenic insert in a single locus within the canola genome.

- The CP4 EPSPS protein in MON 88302 is identical to the CP4 EPSPS protein produced in several other commercially available crops that have been reviewed by USDA and previously deregulated (*e.g.,* Roundup Ready soybean, Roundup Ready 2 Yield soybean, Roundup Ready corn 2, Roundup Ready canola, Roundup Ready sugar beet, Roundup Ready cotton, Roundup Ready Flex cotton and Roundup Ready alfalfa). The safety of CP4 EPSPS proteins present in biotechnology-derived crops has been thoroughly assessed, and is the subject of numerous publications. The mode of action of CP4 EPSPS protein and how it confers glyphosate tolerance has been extensively studied and is well documented in peer reviewed publications.

- A compositional assessment confirmed that MON 88302 seed is compositionally equivalent to seed of conventional canola.

- An extensive evaluation of MON 88302 phenotypic and agronomic characteristics and environmental interactions demonstrated MON 88302 has no increased plant pest potential compared to conventional canola.

- An assessment of potential impact to non-target organisms (NTOs) and endangered species indicated that, under normal agricultural conditions, MON 88302 is unlikely to have adverse effects on these organisms, similar to conventional canola.

- Evaluation of MON 88302 using intended and current cultivation and management practices for canola concluded that deregulation of MON 88302 will not significantly impact canola agronomic practices or land use.

Canola is a Familiar Crop and Ebony is an Appropriate Comparator to MON 88302

Brassica napus is a versatile crop that provides both food and feed to the global economy and whose biology is well understood and documented. There are numerous terms used to describe oil-producing *B. napus* varieties including oilseed rape, rapeseed, rape, low erucic acid rapeseed and canola. For purposes of this petition, *B. napus* will be referred to as oilseed rape and the term canola will be used to denote *B. napus* varieties that produce low (< 2%) erucic acid oil and have levels of glucosinolates below the OECD standard of 30 μmoles/g in meal.

Most U.S. canola production is concentrated in the northern Great Plains where drier, shorter growing seasons make maize and soybean production less attractive. Approximately 1.0 to 1.5M acres of canola are planted annually in eight states with the majority (>85%) being produced in North Dakota. Spring and winter canola varieties have been developed which permit production in both the northern and southern Great Plains, respectively.

Brassica napus is not generally regarded as an environmentally hazardous, colonizing, or invasive species in undisturbed natural ecosystems. Although *B. napus* has some characteristics typical of weedy species such as high reproductive capacity, rapid growth and multiple pollination mechanisms (self, wind, insect), it also has many characteristics typical of domesticated species including low genetic diversity, lack of long-distance seed dispersal mechanisms, limited population persistence, lack of primary seed dormancy and an inability to compete well with perennial species. *Brassica napus* is not listed as an invasive weed in the Catalog of Invasive Plant Species of the U.S., nor is it present on the lists of noxious weed species maintained by the federal government (7 CFR § 360).

The *Brassica napus* canola variety used as the recipient for the DNA insertion to create MON 88302 was Ebony, a non-transgenic conventional spring canola variety. Ebony was used as the conventional canola comparator (referred to in this petition document as the conventional control) in the safety assessment of MON 88302. MON 88302 and the conventional control have similar genetic backgrounds with the exception of the *cp4 epsps* expression cassette.

Molecular Characterization Verified the Integrity and Stability of the Inserted DNA in MON 88302

MON 88302 was developed through *Agrobacterium*-mediated transformation of hypocotyls from canola variety Ebony utilizing plasmid vector PV-BNHT2672. PV-BNHT2672 contains one T-DNA that is delineated by Left and Right Border regions. The T-DNA contains the *cp4 epsps* coding sequence under the control of the *FMV/Tsf1* chimeric promoter, the *Tsf1* leader and intron sequences, and the *E9* 3′ untranslated region. After transformation and subsequent rounds of self-pollination, plants containing only a single T-DNA insertion were identified resulting in production of glyphosate-tolerant canola MON 88302.

Molecular characterization by Southern blot analyses determined that MON 88302 contains one copy of the T-DNA at a single integration locus. These data also demonstrated that MON 88302 does not contain detectable backbone sequences from the plasmid vector. The complete DNA sequence of the insert and adjacent genomic DNA sequences in MON 88302 confirmed the integrity of the inserted *cp4 epsps* expression cassette within the inserted sequences and identified the 5′ and 3′ insert-to-genomic DNA junctions. Southern blot analysis demonstrated that the insert in MON 88302 has been maintained over multiple generations of breeding, thereby confirming the stability of the insert. Further, results from segregation analyses show inheritance and stability of the insert were as expected across multiple generations, which corroborates the molecular insert stability analysis and establishes the genetic behavior of the T-DNA in MON 88302 at a single chromosomal locus.

Data Confirms CP4 EPSPS Protein Safety

A multistep approach was used to characterize and assess the safety of the CP4 EPSPS protein expressed in MON 88302 resulting from the genetic modification. The physicochemical characteristics of the CP4 EPSPS protein in MON 88302 were determined and shown to be equivalent to those of an *E. coli*-produced CP4 EPSPS protein that has been used in CP4 EPSPS protein safety studies. The expression levels of the CP4 EPSPS protein in selected tissues of MON 88302 were determined. An assessment of the allergenic potential of the CP4 EPSPS protein supports the conclusion that the CP4 EPSPS protein does not pose a significant allergenic risk to humans or animals. In addition, the donor organism for the CP4 EPSPS coding sequence, *Agrobacterium* sp. strain CP4, is ubiquitous in the environment and is not commonly known for human or animal pathogenicity or allergenicity. Bioinformatics analysis determined that CP4 EPSPS protein does not share amino acid sequence similarities with known allergens, gliadins, glutenins, or protein toxins. The CP4 EPSPS protein is rapidly digested in simulated digestive fluids and demonstrates no oral toxicity in mice at the level tested. Hence, the consumption of the CP4 EPSPS protein from MON 88302 or its progeny is considered safe for humans and animals.

MON 88302 is Compositionally Equivalent to Conventional Canola

Previous Roundup Ready crops reviewed by the USDA, including the first-generation product Roundup Ready canola (RT73), have had no biologically relevant compositional changes identified, and there is no reason to expect expression of the CP4 EPSPS protein in MON 88302 would affect nutritionally important nutrients, toxicants, and anti-nutrients present in seed from this new product.

Safety assessments of biotechnology-derived crops follow the comparative safety assessment process in which the composition of grain and/or other raw agricultural commodities of the biotechnology-derived crop are compared to the appropriate conventional counterpart that has a history of safe use. Compositional assessments were performed using the principles and analytes outlined in crop-specific OECD consensus documents, in this case for canola composition.

Compositional analysis comparing MON 88302 to the conventional control variety (Ebony) and commercial reference varieties demonstrated that MON 88302 is compositionally equivalent to conventional canola. The background genetics of the conventional control were similar to that of MON 88302, but did not contain the *cp4 epsps* expression cassette. The commercial reference varieties were used to define the natural variability of key nutrients, toxicants, and anti-nutrients in canola varieties that have a history of safe consumption. The samples utilized for compositional analysis were obtained from two U.S. sites and three Canadian sites.

Nutrients assessed in this analysis included proximates (ash, carbohydrates by calculation, moisture, protein, and total fat), fibers (acid detergent fiber [ADF], neutral detergent fiber [NDF], and total dietary fiber [TDF]), amino acids (18 components), fatty acids (FA; C8-C24,), vitamin E (α-tocopherol), and minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc) in seed. The toxicants assessed in seed included erucic acid and glucosinolates (alkyl glucosinolates [including 3-butenyl, 4-pentenyl, 2-hydroxy-3-butenyl, and 2-hydroxy-4-pentenyl glucosinolates], indolyl glucosinolates [including 3-indolylmethyl and 4-hydroxy-3-indoylmethyl], and total glucosinolates). The anti-nutrients assessed in seed included phytic acid and sinapine (as sinapic acid).

Combined-site analyses were conducted to determine statistically significant differences ($\alpha = 0.05$) between MON 88302 and the conventional control seed samples. Statistical results from the combined-site data were evaluated using considerations relevant to the safety and nutritional quality of MON 88302 when compared to the conventional control. Considerations used to assess the relevance of each combined-site statistically significant difference included: 1) the relative magnitude of the difference in the mean values of nutrient, toxicant, and anti-nutrient components between MON 88302 and the conventional control; 2) whether the MON 88302 component mean value is within the range of natural variability of that component as represented by the 99% tolerance interval of the commercial reference varieties grown concurrently in the same trial; 3) evaluation of the reproducibility of the statistically significant ($\alpha = 0.05$) combined-site component differences at individual sites, and 4) an assessment of the differences within

the context of natural variability of commercial canola composition published in the scientific literature. If statistically significant differences detected in the individual site analyses were not observed in the combined-site analysis, they were not considered further for the compositional assessment of safety.

The levels of assessed components in MON 88302 were comparable to those in the conventional control and within the range of variability of commercial reference varieties grown concurrently in the same field trial. Of the 51 components statistically analyzed, 42 were not significantly different from the conventional control. Where statistically significant differences ($\alpha = 0.05$) were observed between MON 88302 and the conventional control in the combined-site analysis, the magnitudes of the differences were small in relation to their natural variability as established by the 99% tolerance interval established from the commercial reference varieties grown concurrently. The mean values for the significantly different components were within the 99% tolerance interval, differences were not observed across all individual sites, and with the exception of alkyl glucosinolates for which a range of values was not available, mean values were within the natural variability of canola components as reported in the scientific literature for conventional canola that have a history of safe consumption. Based on these results, the observed differences were not meaningful to food and feed safety or nutritional value, and led to the conclusion that MON 88302 is compositionally equivalent to conventional canola that has a history of safe consumption.

MON 88302 Does Not Change Canola Plant Pest Potential or Environmental Interactions

Plant pest potential of a biotechnology-derived crop is assessed from the basis of familiarity that the USDA recognizes as an important underlying concept in risk assessment. The concept of familiarity is based on the fact that the biotechnology-derived plant is developed from a conventional plant hybrid or variety whose biological properties and plant pest potential are well known. Familiarity considers the biology of the plant, the introduced trait, the receiving environment, and the interactions among these factors. This provides a basis for comparative risk assessment between a biotechnology-derived plant and the conventional control. Thus, the phenotypic, agronomic, and environmental interaction assessment of MON 88302 included the genetically similar conventional control as a comparator. This evaluation used a weight of evidence approach and considered statistical differences between MON 88302 and the conventional control with respect to reproducibility, magnitude, and directionality. The observations were taken on plants not treated with glyphosate in order to evaluate only the impact of the introduced trait in MON 88302. Comparison to a range of commercial references established the range of natural variability for canola, and provided a context from which to further evaluate any statistical differences. Characteristics assessed included: seed dormancy and germination, pollen morphology, and plant phenotypic observations and environmental interaction evaluations conducted in the field. Commercial references grown concurrently were used to establish a range of natural variability for each assessed characteristic in canola. The phenotypic, agronomic, and environmental interaction assessment demonstrated that MON 88302 is comparable to

the conventional control. Thus, MON 88302 is unlikely to have increased weediness or plant pest potential compared to conventional canola.

Seed dormancy and germination characterization indicated that MON 88302 seed had no changes in the dormancy or germination characteristics that could be indicative of increased plant weediness or pest potential of MON 88302 compared to the conventional control. No statistically significant differences were detected ($\alpha = 0.05$) between MON 88302 and the conventional control for percent viable pollen or pollen grain diameter. Furthermore, no visual differences in general pollen morphology were observed between MON 88302 and the conventional control, demonstrating that the introduction of the glyphosate-tolerance trait did not alter the overall morphology or pollen viability of MON 88302 compared to the conventional control.

The field evaluation of phenotypic, agronomic, and environmental characteristics of MON 88302 also supports the conclusion that MON 88302 is not likely to have increased weediness or plant pest potential or an altered environmental impact compared to conventional canola. The evaluations were conducted at eight field sites in the U.S. and nine field sites in Canada. These 17 field sites provided a diverse range of environmental and agronomic conditions representative of commercial canola production areas in North America. Assessments included 12 phenotypic and agronomic characteristics, as well as observations for plant responses to abiotic stressors and plant-disease and plant-arthropod interactions. The observed phenotypic characteristics were comparable between MON 88302 and the conventional control. Across sites, data show no statistically significant differences between MON 88302 and the conventional control for early stand count, seedling vigor, seed maturity, lodging, plant height, visual rating for pod shattering, quantitative pod shattering, seed quality, yield, and final stand count. Two statistically significant differences were detected between MON 88302 and the conventional control in the combined-site analysis. MON 88302 reached first flowering later than the conventional control (61.1 vs. 56.2 days). However, the mean value of MON 88302 for days to first flowering was within the natural variability of the commercial reference varieties (45.9 – 67.5 days). Therefore, the difference in days to first flower is unlikely to be biologically meaningful in terms of increased weediness potential. MON 88302 also had higher harvested seed moisture than the conventional control (13.2% vs. 11.7%). However, the mean value of MON 88302 for harvested seed moisture was within the natural variability of the commercial reference varieties (7.5% – 14.8%). Therefore, the difference in seed moisture is unlikely to be biologically meaningful in terms of increased weediness potential.

In a qualitative assessment of plant response to abiotic stress, no differences were observed between MON 88302 and the conventional control for 130 out of 131 comparisons involving 9 assessed abiotic stressors. One difference was observed in abiotic stress response in Observation 1 for frost damage at the MBBR site (where MON 88302 was severe and the conventional control was moderate). However, the observed frost damage to MON 88302 was within the range of the damage observed among the commercial reference varieties (slight – severe). In addition, the difference was not observed during any of the other 12 frost damage observations among the sites. Thus, the single difference for frost damage was not considered biologically meaningful

in terms of plant pest potential or an adverse environmental impact from MON 88302 compared to conventional canola.

In a qualitative assessment of plant response to disease damage and arthropod damage, no differences were observed between MON 88302 and the conventional control for any of the 141 comparisons involving 16 assessed diseases or for any of the 165 comparisons for any of the 13 assessed arthropods among all observations at the sites.

In addition, damage by two common pests, flea beetles and seed pod weevil was evaluated quantitatively at four sites. No statistically significant differences were detected between MON 88302 and the conventional control from the four sites evaluated.

In a quantitative assessment of pest and beneficial arthropod abundance, no statistically significant differences were detected between MON 88302 and the conventional control for any of the 51 comparisons, including 36 arthropod pest comparisons and 15 beneficial arthropod comparisons, among the observations at the four sites.

In summary, the phenotypic, agronomic, and environmental interaction data were evaluated to characterize MON 88302, and to assess whether the introduction of the trait in MON 88302 alters the plant pest potential compared to conventional canola. The evaluation, using a weight of evidence approach, considered the reproducibility, magnitude, and direction of detected differences (trends) between MON 88302 and the conventional control and comparison to the range of the commercial reference varieties. Results from the phenotypic, agronomic, and environmental interactions assessment indicated that MON 88302 does not possess weedy characteristics, increased susceptibility or tolerance to specific abiotic stress, diseases, or arthropods, or characteristics that would confer a plant pest risk or a significant environmental impact compared to conventional canola.

MON 88302 Will Not Adversely Affect NTOs or Threatened or Endangered Species

Evaluation of the impacts of MON 88302 on non-target organisms (NTOs) is a component of the plant pest risk assessment. Since MON 88302 does not possess pesticidal activity, all organisms that interact with MON 88302 are considered to be NTOs. The environmental assessment of MON 88302 indicated that it poses no adverse effect on NTOs or endangered species using current and intended agricultural practices. The assessment indicates that the CP4 EPSPS protein found in MON 88302 did not unexpectedly alter plant-arthropod interactions, including beneficial arthropods, or alter disease susceptibility compared to the conventional control.

The safety of the CP4 EPSPS protein present in biotechnology-derived crops has been extensively assessed. A mouse gavage study demonstrated no acute oral toxicity and consequently the low potential for impact to terrestrial vertebrate NTOs including threatened and endangered vertebrate species. In addition, the history of safe use of Roundup Ready crops and the ubiquitous presence of functionally identical EPSPS proteins in plants and microbes in the environment make it unlikely that the presence of CP4 EPSPS protein in MON 88302 will have a significant impact on the receiving

environment, including soil function and water quality. Data from the 2009 North American phenotypic and agronomic study including observational data on environmental interactions such as plant-disease interaction, arthropod damage and arthropod abundance, were collected at select sites for MON 88302 and the conventional control. These results support the conclusion of no adverse environmental impact from cultivation of MON 88302 to non-target organisms and no increased incidence of disease in MON 88302. Taken together, these data support the conclusion that MON 88302 has no reasonable mechanism for harm to NTOs, or impact to threatened and endangered species compared to the cultivation of commercial canola.

Brassica napus is predominantly self-pollinating although interplant (plants that are touching one another) cross pollination rates range from 12% to 55% with a mean of 30%. Most (98.8%) of pollen travels less than twelve meters from its source although dispersal due to pollinators may occur over greater distances at low frequency. *Brassica napus* produces a large amount of pollen that can remain viable up to four to five days under field conditions. This, coupled with the potential for *B. napus* pollen movement, suggests the possibility for hybridization between *B. napus* and related species. Species with which *B. napus* can hybridize under field conditions are found throughout regions in the U.S. where canola is grown. There are reports of hybridization under field conditions with *B. napus* as the pollen donor with six species including *Brassica rapa, Brassica juncea, Brassica oleracea, Hirschfeldia incana, Raphanus raphanistrum,* and *Sinapis arvensis*. In all cases these hybrids had decreased environmental fitness evidenced by a variety of characteristics including decreased pollen viability, seed production, and seedling survival when compared to parental varieties, making gene flow between species unlikely.

Gene flow from *B. napus* canola to *B. napus* vegetables (*e.g.,* Swedes or rutabaga, Siberian kale) is possible as they are members of the same species. Since *B. napus* has chromosomes in common with *B. rapa* and *B. oleracea*, *B. napus* gene flow to *B. rapa* vegetables (*e.g.,* turnip and Chinese cabbage) and *B. oleracea* vegetables (*e.g.,* cabbage, cauliflower, broccoli, collards, kale, Brussels sprouts) is less likely but may occur. However, *B. napus*, *B. rapa* and *B. oleracea* vegetables are not considered weedy, and are generally harvested prior to flowering, preventing cross-pollination, hybridization and seed formation. Thus the potential for *B. napus* gene flow and introgression into closely related vegetable species is low. In the unlikely event that trait introgression and persistence of hybrids between MON 88302 and related species occurred, these plants could be controlled by mechanical or chemical means.

Deregulation of MON 88302 Will Not Significantly Impact Canola Agronomic Practices or Land Use

An assessment of current canola agronomic practices was conducted to determine whether the cultivation of MON 88302 has the potential to impact current canola and weed management practices. Canola fields are typically highly managed agricultural areas that are dedicated to crop production. MON 88302 is likely to be used in common rotations on land previously used for agricultural purposes. Certified seed production will continue to use well-established industry practices to deliver high quality seed with

MON 88302 to growers. Cultivation of MON 88302 is not expected to differ from current canola cultivation using glyphosate-tolerant canola, with the exception of an opportunity to use glyphosate during an expanded window of application and at rates higher than those currently recommended and authorized. Due to the expanded timing of in-crop applications to MON 88302 glyphosate treatments may be later in the growing season than current labeled uses. Monsanto submitted amended labeling to the U.S. Environmental Protection Agency (U.S. EPA) in February 2011 that proposes to modify the current use pattern of glyphosate in canola based on MON 88302.

MON 88302 is similar to conventional canola in its agronomic, phenotypic, ecological, and compositional characteristics and has levels of resistance to insects and diseases comparable to conventional canola. Therefore, no significant impacts on current cultivation and management practices for canola are expected following the introduction of MON 88302. Based on this assessment, the introduction of MON 88302 will not impact current U.S. canola cultivation practices or weed management practices, other than intended weed control benefits.

Conclusion

Based on the data and information presented in this petition, it is concluded that MON 88302 is not likely to be a plant pest. Therefore, Monsanto Company requests a determination from APHIS that MON 88302 and any progeny derived from crosses between MON 88302 and conventional *Brassica* species or deregulated biotechnology-derived crop be granted nonregulated status under 7 CFR Part 340.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS AND DEFINITIONS[1]

~	Approximately
a.e.	Acid Equivalent
a.i.	Active Ingredient
AA	Amino Acid
ADF	Acid Detergent Fiber
AOSA	Association of Official Seed Analysts
Ave	Average
BBCH Scale	Bayer, BASF, Ciba-Geigy and Hoechst Cereal Grain Growth Scale
BLOCKS	A database of amino acid motifs found in protein families
BLOSUM	Blocks Substitution Matrix, used to score similarities between pairs of distantly related protein or nucleotide sequences
BSA	Bovine Serum Albumin
bw	Body Weight
C8-C24	8-24 Carbon-Chain Fatty Acids
CFIA	Canadian Food Inspection Agency
CFR	Code of Federal Regulations
CI	Confidence Interval
cp4 epsps	Codon optimized coding sequence of the *aroA* gene from *Agrobacterium* sp. strain CP4 encoding CP4 EPSPS
CP4 EPSPS	5-Enolpyruvylshikimate-3-phosphate synthase protein from the *Agrobacterium* sp. strain CP4
CPI	Canola Protein Isolate
CSFII	Continuing Surveys of Food Intakes by Individuals
DAP	Days After Planting
DDI	Daily Dietary Intake
DEEM-FCID	Dietary Exposure Evaluation Model-Food Commodity Intake Database
DTT	Dithiothreitol
dw	Dry Weight
E. coli	*Escherichia coli*
EDTA	Ethylenediaminetetraacetic Acid
ELISA	Enzyme-linked Immunosorbent Assay
EPA	Environmental Protection Agency
EPSP	5-enolpyruvylshikimate-3-phosphate
EPSPS	5-enolpyruvylshikimate-3-phosphate synthase enzyme
FA	Fatty Acid
FAO/WHO	Food and Agriculture Organization of the United Nations/World Health Organization
FFDCA	Federal Food, Drug and Cosmetic Act

[1] Alred, G.J., C.T. Brusaw, and W.E. Oliu. 2003. Handbook of Technical Writing, 7th edn., pp. 2-7. Bedford/St. Martin's, Boston, MA.

FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
fw	Fresh Weight
g	g-force
GLP	Good Laboratory Practice
GRAS	Generally Recognized As Safe
HEPES	N-[2-(hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid)]
ILSI	International Life Sciences Institute
kDa	Kilodalton
LB	Laemmli buffer
LOD	Limit of Detection
LOQ	Limit of Quantitation
MALDI-TOF-MS	Matrix Assisted Laser Desorption Ionization - Time of Flight Mass Spectrometry
MMT	Million metric tons
MOE	Margin of Exposure
MW	Molecular Weight
MWCO	Molecular Weight Cutoff
NDF	Neutral Detergent Fiber
NFDM	Non-Fat Dry Milk
NIST	National Institute of Standards and Technology
NOAEL	No Observable Adverse Effect Level
NR	Nitrogen Requirements
NTOs	Non-Target Organisms
OD	Optical Density
OECD	Organization for Economic Co-operation and Development
ORF	Open Reading Frame
OSL	Over Season Leaf
PBS	Phosphate Buffered Saline
PBST	Phosphate Buffered Saline containing 0.05% (v/v) Tween-20
PCC	Previous Crop Credit
PCR	Polymerase Chain Reaction
PEP	Phosphoenolpyruvate
P_i	Inorganic phosphate
PPA	Plant Protection Act
ppm	Parts Per Million
PRESS	Predicted Residual Sum of Squares
PVDF	Polyvinylidene Difluoride
RBD	Refined, Bleached, and Deodorized
RED	Re-registration Eligibility Decision
RfD	Reference Dose
RQ	Risk Quotient
S3P	Shikimate-3-phosphate
Sarkosyl	*N*-lauroylsarcosine, sodium salt
SD	Standard Deviation
SDS-PAGE	Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis
SE	Standard Error

SGF	Simulated Gastric Fluid
sp.	Species
STN	Soil Test Nitrate-nitrogen index
SNP	Single Nucleotide Polymorphism
TDF	Total Dietary Fiber
T-DNA	Transfer DNA
Tm	Melting Temperature
Tz	Tetrazolium
YP	Yield Potential

I. RATIONALE FOR THE DEVELOPMENT OF MON 88302

I.A. Basis for the Request for a Determination of Nonregulated Status under 7 CFR § 340.6

The Animal and Plant Health Inspection Service (APHIS) of the United States (U.S.) Department of Agriculture (USDA) has responsibility, under the Plant Protection Act (Title IV Pub. L. 106-224, 114 Stat. 438, 7 U.S.C. § 7701-7772) to prevent the introduction and dissemination of plant pests into the U.S. APHIS regulation 7 CFR § 340.6 provides that an applicant may petition APHIS to evaluate submitted data to determine that a particular regulated article does not present a plant pest risk and no longer should be regulated. If APHIS determines that the regulated article does not present a plant pest risk, the petition is granted, thereby allowing unrestricted introduction of the article.

Monsanto Company is submitting this request to APHIS for a determination of nonregulated status for the new biotechnology-derived canola product, MON 88302, any progeny derived from crosses between MON 88302 and conventional canola, and any progeny derived from crosses of MON 88302 with biotechnology-derived canola that have previously been granted nonregulated status under 7 CFR Part 340.

I.B. Rationale for the Development of Glyphosate-Tolerant Canola MON 88302

Monsanto Company has developed a second-generation glyphosate-tolerant canola product, MON 88302, designed to provide growers with improved weed control through greater flexibility for glyphosate herbicide application. Weed competition can be a major limiting factor in canola production leading to significant yield reductions (CCC, 2006). Certain perennial weeds, such as Canada thistle, are known to be particularly important to control in canola production. For example, studies have demonstrated that only 10 Canada thistle plants per square meter have resulted in 10% yield loss while 40 plants per square meter have resulted in over 50% yield loss (CCC, 2006). Glyphosate is highly effective against the majority of annual and perennial grasses and broad-leaf weeds including Canada thistle (NDSU, 2005; Padgette et al., 1996). Glyphosate has been shown to have a favorable safety profile by the U.S. EPA (1993a) which has concluded that use of glyphosate will not pose unreasonable risks to humans or the environment.

MON 88302 was produced by incorporation of the *cp4 epsps* coding sequence from the common soil bacterium *Agrobacterium* sp. strain CP4. The *cp4 epsps* coding sequence directs the production of the 5-enolpyruvylshikimate-3-phosphate synthase (termed CP4 EPSPS) that is less sensitive to inhibition by glyphosate compared to the endogenous plant EPSPS. Hence, the CP4 EPSPS renders MON 88302 tolerant to glyphosate, the active ingredient in the Roundup family of agricultural herbicides. The transformation cassette in MON 88302 employs sequences from the promoter of the *TsfI* gene from *Arabidopsis thaliana* and enhancer sequences from the 35S promoter from the figwort mosaic virus (FMV) to enhance CP4 EPSPS production in male reproductive tissues (Feng et al., 2010). Glyphosate is a systemic herbicide and is translocated in the plant generally from a strong source tissue (*e.g.,* leaf) to rapidly developing, or sink tissue

(Devine et al., 1993). Sink tissues, such as pollen, that accumulate glyphosate and lack sufficient CP4 EPSPS expression are considered to be at risk for glyphosate injury. By virtue of enhanced CP4 EPSPS expression in male reproductive tissues, MON 88302 provides tolerance to glyphosate during the sensitive reproductive stages of growth (Feng et al., 2010). Use of MON 88302 will enable growers to apply Roundup herbicide at higher rates and at later stages of development than is possible with the current product facilitating: 1) better control of tough-to-kill weeds; 2) an increased opportunity to control weeds if glyphosate application is delayed due to weather or equipment failure; 3) an enhanced ability to tailor labeled glyphosate applications to weed development stage instead of the canola developmental stage; and 4) enhanced protection of canola plants at more advanced development stages. Use of MON 88302 will provide growers with the opportunity to ensure weeds that may impact yields are removed at the optimal time while minimizing the potential for crop injury.

I.C. Submissions to Other Regulatory Agencies

Under the Coordinated Framework for Regulation of Biotechnology, the responsibility for regulatory oversight of biotechnology-derived crops falls primarily on three U.S. agencies: U.S. Food and Drug Administration (FDA), the United States Department of Agriculture (USDA), and in the case of plant incorporated protectants, the Environmental Protection Agency (EPA). Deregulation of MON 88302 by USDA constitutes only one component of the overall regulatory oversight and review of this product. As a practical matter, MON 88302 cannot be released and marketed until FDA and USDA have completed their reviews and assessments under their respective jurisdictions.

I.C.1. Submission to the FDA

MON 88302 falls within the scope of the 1992 FDA policy statement concerning regulation of products derived from new plant varieties, including those developed through biotechnology (U.S. FDA, 1992). In compliance with this policy, Monsanto has initiated a consultation with the FDA (BNF No. 127) on the food and feed safety and compositional assessment of MON 88302. Monsanto submitted a safety and nutritional assessment summary document to the FDA in March 2011.

I.C.2. Submission to the EPA

The EPA has authority over the use of pesticide substances under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), as amended (7 U.S.C § 136 *et seq*.). Monsanto submitted amended labeling to the U.S. Environmental Protection Agency (U.S. EPA) in February 2011 for EPA Registration Numbers 524-537 (Roundup WeatherMAX® Herbicide) and 524-549 (Roundup PowerMAX® Herbicide), that propose to modify the current use pattern of glyphosate in canola based on MON 88302. The post emergence (in-crop) use of glyphosate in Roundup Ready canola was first approved by the U.S. EPA in March 1999. Although the amended labeling increases the rate of application and widens the application period relative to canola development, this use of glyphosate does not present any new environmental exposure scenarios not previously evaluated for use

on other Roundup Ready crops which have already been deemed acceptable by the U.S. EPA.

I.C.3. Submissions to Foreign Government Agencies

To support commercial introduction of MON 88302 in the U.S., regulatory submissions will be made to countries that import significant quantities of canola and canola products from the U.S. This results in submissions to a number of additional governmental regulatory agencies including, but not limited to the Ministry of Agriculture, People's Republic of China; Japan's Ministry of Agriculture, Forestry, and Fisheries and the Ministry of Health, Labor, and Welfare; the Canadian Food Inspection Agency and Health Canada; the Intersectoral Commission for Biosafety of Genetically Modified Organisms, Mexico; the European Food Safety Authority, Food Standards Australia New Zealand, the Korea Food and Drug Administration, and the Rural Development Administration of Korea, as well as to regulatory authorities in other canola importing countries with functioning regulatory systems. As appropriate, notifications will be made to countries that import significant quantities of canola and canola products and do not have a formal regulatory review process for biotechnology-derived crops.

II. THE BIOLOGY OF CANOLA

Brassica napus L. is a versatile crop that provides both food and feed to the global economy, and whose biology is well understood and documented. There are numerous terms used to describe oil-producing *B. napus* varieties including oilseed rape, rapeseed, rape, low erucic acid rapeseed and canola. For purposes of this document, *B. napus* will be referred to as oilseed rape and the term canola will be used to denote *B. napus* varieties that produce low (< 2%) erucic acid oil and have levels of glucosinolates below the accepted standard of 30 µmoles/g in meal (OECD, 2001).

The Organisation for Economic Co-operation and Development Consensus Document on the Biology of *Brassica napus* (OECD, 1997) provides key information on:

- general description of *B. napus* biology, including taxonomy and morphology and use of *B. napus* as a crop plant

- agronomic practices in *B. napus* cultivation

- geographic centers of origin

- reproductive biology

- inter-species/genus introgression into relatives and interactions with other organisms

- summary of the ecology of *B. napus*

Additional information on the biology of *B. napus* can be found on the Canadian Food Inspection Agency website (CFIA, 2005), and the Australian Government Department of Health and Ageing (Office of the Gene Technology Regulator) website (OGTR, 2008). Information on the taxonomy of *B. napus* can be found in the U.S. Department of Agriculture Natural Resources Conservation Service PLANTS database (USDA-NRCS, 2010b).

To support the evaluation of the plant pest potential of MON 88302 relative to conventional canola, additional information regarding several aspects of canola biology can be found elsewhere in this petition. This includes: agronomic practices for canola in Section VIII; volunteer management of canola in Section VIII; and inter-species/genus introgression potential in Section IX.

II.A. Canola as a Crop

While canola oil can be derived from any one of three species: *Brassica napus, Brassica rapa,* and *Brassica juncea* (OGTR, 2008; U.S. FDA, 1988; 2000), most canola oil is derived from *Brassica napus*. Oilseed rape is a member of the mustard (Brassicaceae) family, and has been cultivated by ancient civilizations in Asia and the Mediterranean primarily for its use as oil in lamps (Colton and Sykes, 1992). Later *B. napus* oil was used as an industrial lubricant, and today there is still demand for high erucic oil in a variety of industrial applications.

Until recently, the presence of the naturally occurring toxicants, erucic acid in the oil fraction and glucosinolates in the meal has made rapeseed oil and meal derived from *B. napus* unattractive for human consumption and as an animal feed, respectively, particularly in western countries. High erucic acid rapeseed oil (as much as 50% of total fatty acids) has been shown to have cardiopathic potential resulting in a weakening of the heart muscle in experimental animals (Bozcali et al., 2009; Chien et al., 1983) and high levels of glucosinolates made oilseed rape meal unsuitable for use in animal nutrition because of anti-nutritional, goitrogenic (suppresses thyroid function), reproductive, and palatability problems (Fenwick et al., 1989). However, in the 1960s intensive breeding programs resulted in the development and introduction of low erucic acid or canola (Canadian oil, low acid) varieties of oilseed rape (OECD, 2001; OGTR, 2008). At approximately the same time low erucic acid varieties of *B. rapa* were introduced (OECD, 2001). Slightly later, in the 1980s, low erucic acid varieties of *B. juncea* were developed (CCC, 1999). However, *B. napus* varieties are the most commonly grown canola oil-producing varieties in the U.S. (Boyles et al., 2009).

Brassica napus, an amphidiploid (chromosome n=19, AA and CC genomes), is thought to be derived from a cross between two diploid *Brassica* species, *B. rapa* (chromosome n=10, AA genome) and *B. oleracea* (chromosome n=9, CC genome). *Brassica napus* has the greatest sexual compatibility with *B. rapa* and *B. juncea* under natural field conditions, but has also been known to outcross with some wild relatives including *Raphanus raphanistrum* (wild radish) and *Hirschfeldia incana* (shortpod mustard) (OECD, 1997; OGTR, 2008).

There are spring and winter biotypes of canola varieties. Spring canola, a cool season crop, is grown in Canada, southern Australia, northern China, and in the northern Great Plains region of the U.S. Spring canola is slow growing and does not compete well with weeds in its early growth stages. Closely related weeds like wild mustard, stinkweed and shepherd's purse are often problematic in commercial spring canola fields, and weeds must be controlled early in the spring canola life cycle to avoid yield loss due to competition (OECD, 1997). Winter canola is planted in the fall, requires vernalization (exposure to winter cold) to flower, and is grown in parts of Europe, Asia, the northwestern U.S. and in the central portions of the U.S. Great Plains. Winter canola, once established, suppresses and out-competes most annual weeds (Boyles et al., 2009). In addition to various pre-emergent weed control options, varieties of canola having tolerance to glyphosate, glufosinate and imazamox herbicides for weed control in canola fields are widely available.

Canola oil is currently the world's third largest source of vegetable oil (15%) after soybean oil (28%) and palm oil (32%) (ASA, 2010; USDA-ERS, 2010). Canola oil appeals to health conscious consumers because it has the lowest level of saturated fat of all edible oils, the second-highest level of monounsaturated fat, and is free of artificial trans-fatty acids (Brown et al., 2008; USDA-ERS, 2010). Canola seed is also processed into canola meal which is used as high protein animal feed. Canola meal is the second largest protein meal produced in the world. However, it is relatively small compared to soybean meal. Global production of canola meal was 30.8 million metric tons (MMT) in 2008/2009 compared to 151.6 MMT for soybean meal (USDA-ERS, 2010). Canola meal

contains less protein than soybean meal (34-38% vs. 44-49%) and fewer key amino acids (USDA-ERS, 2010).

The United States produced only about 0.7 MMT (1.1%) of the world's canola production (58 MMT) in the growing season 2008/2009 (USDA-FAS, 2010). The European Union, Canada, and China were the largest producers of canola with 19.0, 12.6, and 12.1 MMT, respectively.

II.B. Characteristics of the Recipient Plant

The *B. napus* canola variety used as the recipient for the DNA insertion to create MON 88302 was Ebony, a non-transgenic conventional spring canola variety registered with the Canadian Food Inspection Agency in 1994 by Monsanto Company (CFIA, 2010). Ebony originated from a cross of varieties (Bienvenu × Alto) × Cesar. Selection criteria for the non-transgenic variety included yield, oil and protein content, and tolerance to the fungus *Leptosphaeria maculans*, commonly known as blackleg (CFIA, 1994). Ebony was used to produce the glyphosate-tolerant canola MON 88302 because it responds well to *Agrobacterium*-mediated transformation and tissue regeneration.

II.C. Canola as a Test System in Product Safety Assessment

Ebony was used as the conventional canola comparator (referred to in this document as the conventional control) in the safety assessment of MON 88302. The background genetics of the conventional control are similar to that of MON 88302, but do not contain the *cp4 epsps* expression cassette. In addition, commercial conventional canola varieties (referred to in this consultation document as commercial reference varieties) were used to establish ranges of natural variability or responses representative of commercial canola varieties. The commercial reference varieties used at each location were selected based on their availability and agronomic fit for the geographic region.

III. DESCRIPTION OF THE GENETIC MODIFICATION

MON 88302 was developed through *Agrobacterium*-mediated transformation of hypocotyls from Ebony canola variety utilizing plasmid vector PV-BNHT2672. This section describes the plasmid vector, the donor gene, and the regulatory elements used in the development of MON 88302 as well as the deduced amino acid sequence of the CP4 EPSPS protein produced in MON 88302. In this section, transfer DNA (T-DNA) refers to DNA that is transferred to the plant during transformation. An expression cassette is comprised of sequences to be transcribed and the regulatory elements necessary for the expression of those sequences.

III.A. The Plasmid Vector PV-BNHT2672

PV-BNHT2672 was used in the transformation of canola to produce MON 88302 and is shown in Figure III-1. The elements included in this plasmid vector are described in Table III-1. PV-BNHT2672 is approximately 9.7 kb and contains one T-DNA that is delineated by Left Border and Right Border regions. The T-DNA contains the *cp4 epsps* coding sequence under the control of the *FMV/Tsf1* chimeric promoter, the *Tsf1* leader and intron sequences, and the *E9* 3′ untranslated region. The chloroplast transit peptide CTP2 directs transport of the CP4 EPSPS protein to the chloroplast and is derived from *CTP2* target sequence of the *Arabidopsis thaliana shkG* gene.

The backbone sequence of PV-BNHT2672, located outside of the T-DNA, contains two origins of replication for maintenance of plasmid vector in bacteria (*ori V* and *ori-pBR322*), a bacterial selectable marker gene (*aadA*), and a coding sequence for repressor of primer protein (*rop*) for maintenance of plasmid vector copy number in *Escherichia coli (E. coli)*. A description of the genetic elements and their prefixes (*e.g.,* B-, P-, L-, I-, TS-, CS-, T-, and OR-) in PV-BNHT2672 is provided in Table III-1.

III.B. Description of the Transformation System

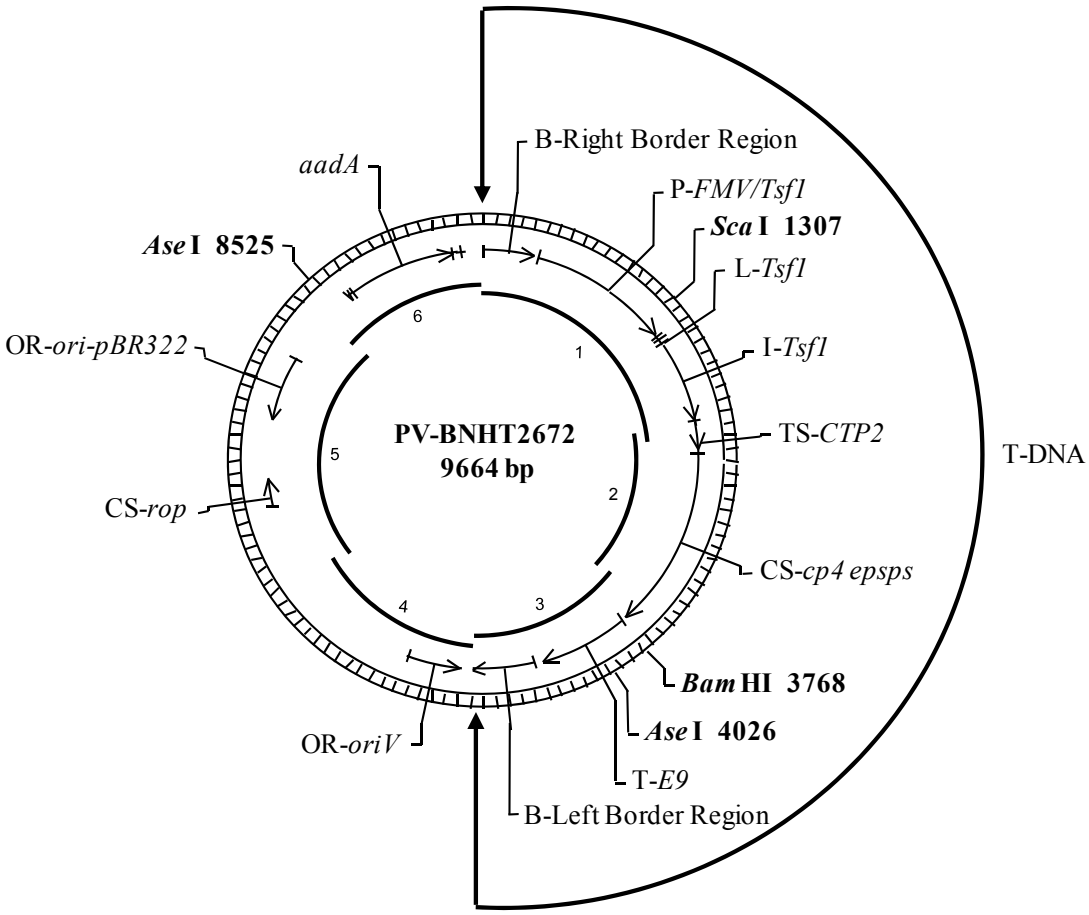
MON 88302 was developed through *Agrobacterium*-mediated transformation of canola hypocotyls, based on the method described by Radke et al. (1992), utilizing PV-BNHT2672 (Figure III-1). In summary, hypocotyl segments were excised from Ebony seedlings grown in the dark. After co-culturing with *Agrobacterium* carrying the vector, the hypocotyl segments were placed on callus growth medium that contained carbenicillin, ticarcillin disodium and clavulanate potassium to inhibit the growth of excess *Agrobacterium*. The hypocotyls were then placed in selection media containing glyphosate to inhibit the growth of untransformed cells and plant growth regulators conducive to shoot regeneration. Rooted R_0 plants with normal phenotypic characteristics were selected and transferred to soil for growth and further assessment.

The R_0 plants generated through the *Agrobacterium*-mediated transformation were transferred to soil for growth and then self pollinated to produce R_1 seed. R_0 and R_1 plants were evaluated for tolerance to glyphosate and screened for the presence of the T-DNA (*cp4 epsps* expression cassette) and absence of backbone sequence (*ori V*). Subsequently, the *cp4 epsps* homozygous R_1 plant was self-pollinated to give rise to R_2

plants. Homozygous R_2 plants containing one copy of the T-DNA inserted at a single locus, were identified by a combination of analytical techniques including glyphosate spray, polymerase chain reaction (PCR), and Southern blot analysis, resulting in production of glyphosate-tolerant canola MON 88302. MON 88302 was selected as the lead event based on superior phenotypic characteristics and its comprehensive molecular profile. Regulatory studies on MON 88302 were initiated to further characterize the genetic insertion and the expressed protein, and to establish the food, feed, and environmental safety relative to conventional canola. The major steps involved in the development of MON 88302 are depicted in Figure III-2.

III.C. The *cp4 epsps* Coding Sequence and the CP4 EPSPS Protein

The *cp4 epsps* expression cassette, or T-DNA in this petition, encodes a 47.6 kDa CP4 EPSPS protein consisting of a single polypeptide of 455 amino acids (Figure III-3) (Padgette et al., 1996). The *cp4 epsps* coding sequence is the codon optimized coding sequence of the *aroA* gene from *Agrobacterium* sp. strain CP4 encoding CP4 EPSPS (Barry et al., 2001; Padgette et al., 1996). The CP4 EPSPS protein is similar and functionally identical to endogenous plant EPSPS enzymes, but has a much reduced affinity for glyphosate, the active ingredient in Roundup agricultural herbicides, relative to endogenous plant EPSPS (Barry et al., 2001; Padgette et al., 1996).



Probe	DNA Probe	Start Position (bp)	End Position (bp)	Total Length (kb)
1	T-DNA Probe 1	1	2287	~2.3
2	T-DNA Probe 2	2231	3618	~1.4
3	T-DNA Probe 3	3562	4910	~1.3
4	Backbone Probe 4	4911	6564	~1.7
5	Backbone Probe 5	6512	8383	~1.9
6	Backbone Probe 6	8329	9664	~1.3

Figure III-1. Circular Map of PV-BNHT2672 Showing Probes 1-6
A circular map of PV-BNHT2672 used to develop MON 88302 is shown. Genetic elements and restriction sites (in bold) used in Southern analyses (with positions relative to the first base pair of the plasmid vector) are shown on the exterior of the map. The probes used in the Southern analyses are shown on the interior of the map and listed in the table. PV-BNHT2672 contains a single T-DNA.

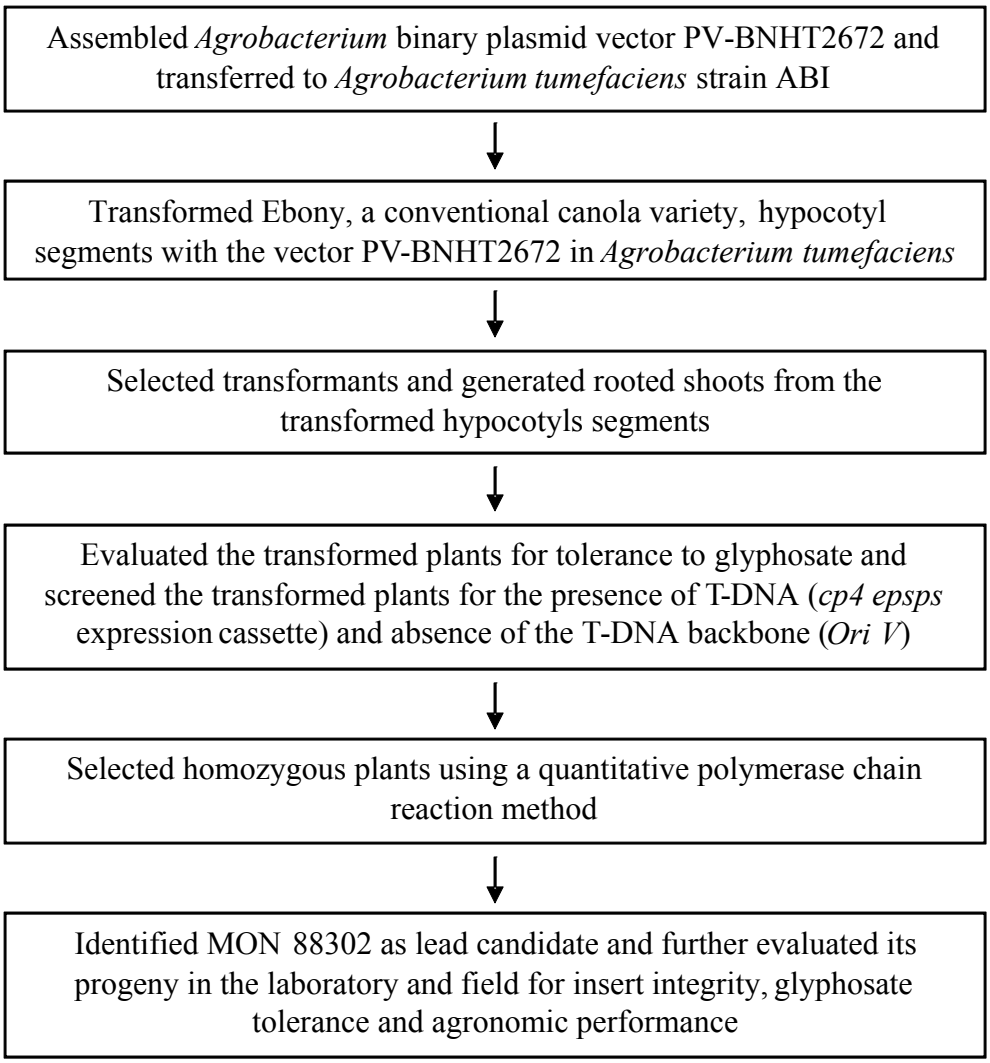


Figure III-2. Schematic of the Development of MON 88302

III.D. Regulatory Sequences

The *cp4 epsps* coding sequence in MON 88302 is under the regulation of the *FMV/Tsf1* chimeric promoter, the *Tsf1* leader and intron sequences, and the *E9* 3′ untranslated region. The *FMV/Tsf1* chimeric promoter, which directs transcription in plant cells, contains enhancer sequences from the promoter of the FMV 35S RNA (Richins et al., 1987) combined with the promoter from the *Tsf1* gene of *Arabidopsis thaliana* that encodes elongation factor EF-1α (Axelos et al., 1989). The *Tsf1* leader sequence is the 5' untranslated region from the *Tsf1* gene of *Arabidopsis thaliana* (Axelos et al., 1989). The *E9* 3' untranslated region is the 3' untranslated region of the pea (*Pisum sativum*) ribulose-1,5-bisphosphate carboxylase small subunit (*rbcS2*) *E9* gene (Coruzzi et al., 1984) and is present to direct polyadenylation of the *cp4 epsps* transcript. The chloroplast transit peptide CTP2 directs transport of the CP4 EPSPS protein to the chloroplast and is derived from *CTP2* target sequence of the *Arabidopsis thaliana shkG* gene (Herrmann, 1995; Klee et al., 1987).

III.E. T-DNA Borders

PV-BNHT2672 contains Right Border and Left Border regions (Figure III-1 and Table III-1) that were derived from *Agrobacterium tumefaciens* plasmids. The border regions each contain a 24-25 bp nick site that is the site of DNA exchange during transformation (Barker et al., 1983; Depicker et al., 1982; Zambryski et al., 1982). The border regions separate the T-DNA from the plasmid backbone region and are involved in the efficient transfer of the T-DNA into the canola genome.

III.F. Genetic Elements Outside of the T-DNA Borders

Genetic elements that exist outside of the T-DNA border regions are those that are essential for the maintenance or selection of PV-BNHT2672 in bacteria. The origin of replication, *ori V,* is required for the maintenance of the plasmid in *Agrobacterium* and is derived from the broad host plasmid RK2 (Stalker et al., 1981). The origin of replication, *ori-pBR322,* is required for the maintenance of the plasmid in *E. coli* and is derived from the plasmid vector pBR322 (Sutcliffe, 1979). Coding sequence *rop* encodes the repressor of primer (ROP) protein which is necessary for the maintenance of plasmid copy number in *E. coli* (Giza and Huang, 1989). The selectable marker *aadA* is a bacterial promoter and coding sequence for an enzyme from transposon Tn7 that confers spectinomycin and streptomycin resistance (Fling et al., 1985) in *E. coli* and *Agrobacterium* during molecular cloning. As these elements are outside the border regions, they are not expected to be transferred into the canola genome. The absence of the backbone sequence in MON 88302 has been confirmed by Southern blot analyses (see Section IV.B).

Table III-1. Summary of Genetic Elements in PV-BNHT2672

Genetic Element	Location in Plasmid	Function (Reference)
T-DNA		
B[1]-Right Border Region	1-357	DNA region from *Agrobacterium tumefaciens* containing the Right Border sequence used for transfer of the T-DNA (Depicker et al., 1982; Zambryski et al., 1982)
Intervening Sequence	358-427	Sequence used in DNA cloning
P[2]-*FMV/Tsf1*	428-1467	Chimeric promoter consisting of the promoter of the *Tsf1* gene from the *Arabidopsis thaliana* encoding elongation factor EF-1α (Axelos et al., 1989) and enhancer sequences from the 35S promoter from the figwort mosaic virus (Richins et al., 1987)
L[3]-*Tsf1*	1468-1513	5' untranslated leader (exon 1) from the *Arabidopsis thaliana Tsf1* gene encoding elongation factor EF-1α (Axelos et al., 1989)
I[4]-*Tsf1*	1514-2135	Intron from the *Arabidopsis thaliana Tsf1* gene encoding elongation factor EF-1α (Axelos et al., 1989)
Intervening Sequence	2136-2144	Sequence used in DNA cloning
TS[5]-*CTP2*	2145-2372	Targeting sequence from the *shkG* gene encoding the chloroplast transit peptide region of *Arabidopsis thaliana* EPSPS (Herrmann, 1995; Klee et al., 1987) that directs transport of the CP4 EPSPS protein to the chloroplast
CS[6]-*cp4 epsps*	2373-3740	Codon optimized coding sequence of the *aroA* gene from the *Agrobacterium* sp. strain CP4 encoding the CP4 EPSPS protein (Barry et al., 2001; Padgette et al., 1996)
Intervening Sequence	3741-3782	Sequence used in DNA cloning
T[7]-*E9*	3783-4425	3' untranslated sequence from the *rbcS2* gene of *Pisum sativum* (pea) encoding the Rubisco small subunit (Coruzzi et al., 1984)
Intervening Sequence	4426-4468	Sequence used in DNA cloning
B-Left Border Region	4469-4910	DNA region from *Agrobacterium tumefaciens* containing the Left Border sequence used for transfer of the T-DNA (Barker et al., 1983; Zambryski et al., 1982)

Table III-1. Summary of Genetic Elements in PV-BNHT2672 (continued)

Genetic Element	Location in Plasmid	Function (Reference)
Vector Backbone		
Intervening Sequence	4911-4996	Sequence used in DNA cloning
OR[8]- *oriV*	4997-5393	Origin of replication from the broad host range plasmid RK2 for maintenance of plasmid in *Agrobacterium* (Stalker et al., 1981)
Intervening Sequence	5394-6901	Sequence used in DNA cloning
CS-*rop*	6902-7093	Coding sequence for repressor of primer protein for maintenance of plasmid copy number in *E. coli* (Giza and Huang, 1989)
Intervening Sequence	7094-7520	Sequence used in DNA cloning
OR-*ori-pBR322*	7521-8109	Origin of replication from pBR322 for maintenance of plasmid in *E. coli* (Sutcliffe, 1979)
Intervening Sequence	8110-8639	Sequence used in DNA cloning
aadA	8640-9528	Bacterial promoter, coding sequence, and 3′ untranslated region for an aminoglycoside-modifying enzyme, 3"(9)-*O*-nucleotidyl-transferase from the transposon Tn7 (Fling et al., 1985) that confers spectinomycin and streptomycin resistance
Intervening Sequence	9529-9664	Sequence used in DNA cloning

[1] B, Border
[2] P, Promoter
[3] L, Leader
[4] I, Intron
[5] TS, Targeting Sequence
[6] CS, Coding Sequence
[7] T, Transcription Termination Sequence
[8] OR, Origin of Replication

```
  1   MAQVSRICNG VQNPSLISNL SKSSQRKSPL SVSLKTQQHP RAYPISSSWG
 51   LKKSGMTLIG SELRPLKVMS SVSTACMLHG ASSRPATARK SSGLSGTVRI
101   PGDKSISHRS FMFGGLASGE TRITGLLEGE DVINTGKAMQ AMGARIRKEG
151   DTWIIDGVGN GGLLAPEAPL DFGNAATGCR LTMGLVGVYD FDSTFIGDAS
201   LTKRPMGRVL NPLREMGVQV KSEDGDRLPV TLRGPKTPTP ITYRVPMASA
251   QVKSAVLLAG LNTPGITTVI EPIMTRDHTE KMLQGFGANL TVETDADGVR
301   TIRLEGRGKL TGQVIDVPGD PSSTAFPLVA ALLVPGSDVT ILNVLMNPTR
351   TGLILTLQEM GADIEVINPR LAGGEDVADL RVRSSTLKGV TVPEDRAPSM
401   IDEYPILAVA AAFAEGATVM NGLEELRVKE SDRLSAVANG LKLNGVDCDE
451   GETSLVVRGR PDGKGLGNAS GAAVATHLDH RIAMSFLVMG LVSENPVTVD
501   DATMIATSFP EFMDLMAGLG AKIELSDTKA A
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Figure III-3. Deduced Amino Acid Sequence of the CP4 EPSPS Precursor Protein
The amino acid sequence of the CP4 EPSPS precursor protein was deduced from the full-length coding nucleotide sequence present in PV-BNHT2672. The 76 amino acid CTP2, the transit peptide of the *Arabidopsis thaliana* EPSPS protein, is underlined. CTP2 targets CP4 EPSPS protein to the chloroplasts. At the chloroplast the CTP2 is cleaved producing the mature 455 amino acid CP4 EPSPS protein that begins with the methionine at position 77.

IV. CHARACTERIZATION OF THE GENETIC MODIFICATION

Characterization of the DNA insert in MON 88302 was conducted by Southern blot, PCR and DNA sequence analyses. The results of this characterization demonstrate that MON 88302 contains a single copy of the *cp4 epsps* expression cassette, *i.e.*, the T-DNA, is stably integrated at a single locus and is inherited according to Mendelian principles over multiple generations. These conclusions were based on several lines of evidence: 1) Southern blot analyses assayed the entire canola genome for the presence of T-DNA and the absence of the backbone sequences derived from PV-BNHT2672, and demonstrated that only a single copy of the T-DNA was inserted at a single site; 2) DNA sequence analyses determined the exact sequence of the inserted DNA and the DNA sequences flanking the 5' and 3' ends of the insert, and allowed a comparison of the T-DNA sequence to the plasmid vector to confirm that only the expected sequences were integrated; 3) DNA sequences flanking the 5' and 3' ends of the insert were compared to the sequence of the insertion site in conventional canola to identify any rearrangements that occurred at the insertion site during transformation;. 4) Southern blot analysis demonstrated that the insert in MON 88302 has been maintained over multiple generations of breeding, thereby confirming the stability of the insert; 5) segregation analyses show inheritance and stability of the insert were as expected across multiple generations. Taken together, the characterization of the genetic modification demonstrates that a single copy of the T-DNA was inserted at a single locus of the canola genome and that no plasmid vector backbone sequences are present in MON 88302.

Southern blot analyses were used to determine the copy number and insertion sites of the integrated DNA as well as the presence or absence of plasmid vector backbone sequences. The Southern blot strategy was designed to ensure that all potential transgenic segments would be identified. The entire canola genome was assayed with probes that spanned the complete plasmid vector to detect the presence of the insert as well as confirm the absence of any plasmid vector backbone sequences. This was accomplished by using probes that were not more than 2.5 kb in length to ensure a high level of sensitivity. This high level of sensitivity was demonstrated for each blot by detection of a positive control added at 0.1 genome equivalent. Two sets of restriction enzymes were specifically chosen to fully characterize the T-DNA and detect any potential fragments of the T-DNA and backbone sequences. The restriction enzyme sets were chosen such that each enzyme set cleaves once within the inserted T-DNA and at least once within the known DNA flanking the 5' or 3' end of the insert. As a consequence, at least one segment containing a portion of the insert with the adjacent 5' flanking DNA generated by one set of the enzyme(s) is of a predictable size and overlaps with another predictable size segment containing a portion of the insert with the adjacent 3' flanking DNA generated by another set of the enzyme(s). This two-set-enzyme design ensures that the entire insert is identified in a predictable hybridization pattern. This strategy also maximizes the possibility of detecting an insertion elsewhere in the genome that could be overlooked if that the fragment co-migrated on the gel with an expected fragment.

To determine the number of copies and insertion sites of the T-DNA, and the presence or absence of the plasmid vector backbone sequences, duplicated samples that consisted of

equal amounts of digested DNA were run on the agarose gel. One set of samples was run for a longer period of time (long run) than the second set (short run). The long run allows for greater resolution of large molecular weight DNA, whereas the short run allows for retaining the small molecular weight DNA on the gel. The molecular weight markers on the left of the figures were used to estimate the sizes of the bands present in the long run lanes of the Southern blots, and the molecular weight markers on the right of the figures were used to estimate the sizes of bands present in the short run lanes of the Southern blots (Figure IV-2 through Figure IV-6). Southern blot analyses determined that a single copy of the T-DNA was inserted at a single locus of the canola genome, and no additional genetic elements, including backbone sequences, from PV-BNHT2672 were detected in MON 88302.

PCR and DNA sequence analyses complement the Southern analyses. PCR and DNA sequence analyses performed on MON 88302 determined the complete DNA sequence of the insert and flanking genomic DNA sequences in MON 88302, confirmed the predicted organization of the genetic elements within the insert, and determined the sequences flanking the insert. In addition, DNA sequence analyses confirmed that each genetic element in the insert is intact and the sequence of the insert is identical to the corresponding sequence in PV-BNHT2672. Furthermore, genomic organization at the MON 88302 insertion site was determined by comparing the 5' and 3' flanking sequences of the insert to the sequence of the insertion site in conventional canola.

The stability of the T-DNA present in MON 88302 across multiple generations was demonstrated by Southern blot fingerprint analysis. Genomic DNA from multiple generations of MON 88302 (Figure IV-9) was digested with one of the enzyme sets used for the insert and copy number analyses and was hybridized with two probes that detect restriction segments that encompass the entire insert. This fingerprint strategy consists of two insert segments each containing its adjacent genomic DNA that assesses not only the stability of the insert, but also the stability of the DNA directly adjacent to the insert.

Segregation analysis was conducted to determine the inheritance and stability of the T-DNA insert in MON 88302. Results from this analysis demonstrated the inheritance and stability of the insert were as expected across multiple generations (Figure IV-11, Table IV-3), which corroborates the molecular insert stability analysis and establishes the genetic behavior of the T-DNA at a single chromosomal locus.

The Southern blot analyses confirmed that the T-DNA reported in Figure IV-1 represents the only detectable insert in MON 88302. A circular map of PV-BNHT2672 annotated with the probes used in the Southern blot analysis is presented in Figure III-1 and the genetic elements within the MON 88302 insert are summarized in Table IV-2. A linear map depicting restriction sites within the insert as well as within the DNA immediately flanking the insert in MON 88302 is shown in Figure IV-1. Based on the plasmid map and the linear map of the insert, a table summarizing the expected DNA segments for Southern analyses is presented in Table IV-1. The results from the Southern blot analyses are presented in Figure IV-2 through Figure IV-6. PCR amplification of the MON 88302 insert and the insertion site in the conventional control (Ebony) for DNA sequence analysis are shown in Figure IV-7 and Figure IV-8, respectively. The

generations used in the generational stability analysis are depicted in the breeding history shown in Figure IV-9 and the results from the generational stability analysis are presented in Figure IV-10. The breeding path for generating the segregation data is shown in Figure IV-11 and the results for the segregation analysis are presented in Table IV-3. Materials and methods used for the characterization of the insert in MON 88302 are found in Appendix B.

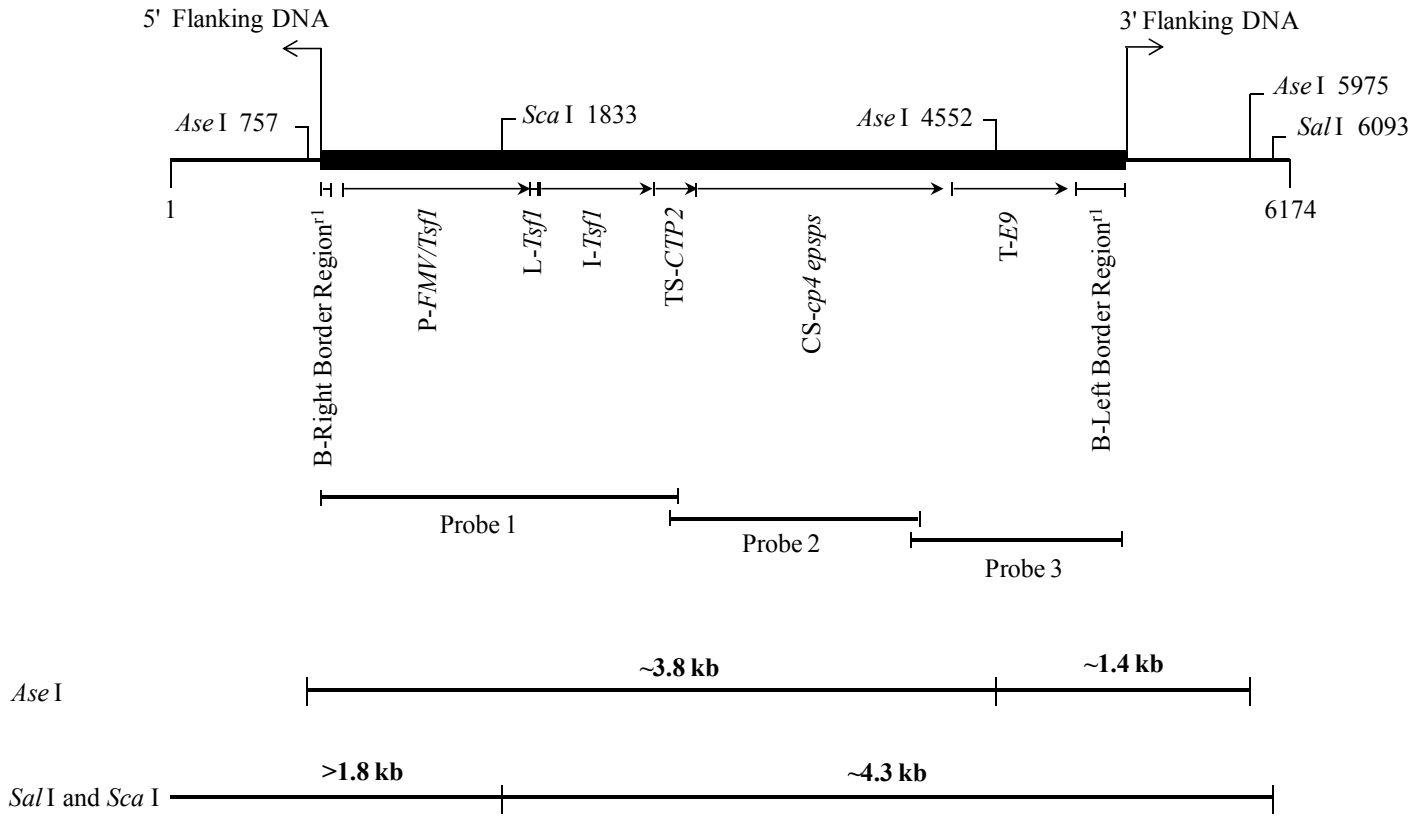


Figure IV-1. Schematic Representation of the Insert and Flanking DNA in MON 88302

A linear map of the insert and DNA flanking the insert in MON 88302 is shown. Right-angled arrows indicate the ends of the integrated T-DNA and the beginning of the flanking DNA. Identified on the linear map are genetic elements within the insert, as well as the sites of the restriction enzymes used in the Southern analyses with positions relative to the first base pair of the DNA sequence represented in this map. The relative sizes and locations of the T-DNA probes and the expected sizes of restriction fragments are indicated in the lower portion of the scheme. This schematic diagram is not drawn to scale. Locations of genetic elements and T-DNA probes are approximate. Probes are also shown in Figure III-1.

Table IV-1. Summary Chart of the Expected DNA Segments Based on Hybridizing Probes and Restriction Enzymes Used in MON 88302 Analysis

Southern Blot Analysis		**T-DNA**		**Backbone**			**Insert Stability**
Figure Number		IV-2	IV-3	IV-4	IV-5	IV-6	IV-10
Probe Used		1, 3	2	4	5	6	1, 3
Probing Target	**Digestion Enzyme**	**Expected Band Sizes on Each Southern Blot**					
Plasmid PV-BNHT2672	*Bam* HI and *Sca* I	~2.5 kb ~7.2 kb	~2.5 kb	~7.2 kb	~7.2 kb	~7.2 kb	~2.5 kb ~7.2 kb
Probe Template Spikes[1]	N/A	~2.3 kb ~1.3 kb	~~[2]	~~[2]	~~[2]	~~[2]	~2.3 kb ~1.3 kb
MON 88302	*Ase* I	~3.8 kb ~1.4 kb	~3.8 kb	No band	No band	No band	~3.8 kb ~1.4 kb
	Sal I and *Sca* I	>1.8 kb ~4.3 kb	~4.3 kb	No band	No band	No band	--[3]

[1] probe template spikes were used as positive hybridization controls in Southern blot analyses when multiple probes were hybridized to the Southern blot simultaneously.

[2] '~~' indicates that probe template spikes were not used.

[3] '--' indicates that the combination of the restriction enzymes was not used in the analysis.

Table IV-2. Summary of Genetic Elements in MON 88302

Genetic Element	Location in Sequence	Function (Reference)
5' Flanking Sequence	1-839	DNA sequence adjacent to the 5' end of the insertion site
B[1]-Right Border Region [r1]	840-882	DNA region from *Agrobacterium tumefaciens* containing the Right Border sequence used for transfer of the T-DNA (Depicker et al., 1982; Zambryski et al., 1982)
Intervening Sequence	883-952	Sequence used in DNA cloning
P[2]-*FMV/Tsf1*	953-1992	Chimeric promoter consisting of the promoter of the *Tsf1* gene from the *Arabidopsis thaliana* encoding elongation factor EF-1α (Axelos et al., 1989) and enhancer sequences from the 35S promoter from the figwort mosaic virus (Richins et al., 1987)
L[3]-*Tsf1*	1993-2038	5' untranslated leader (exon 1) from the *Arabidopsis thaliana Tsf1* gene encoding elongation factor EF-1 α (Axelos et al., 1989)
I[4]-*Tsf1*	2039-2660	Intron from the *Arabidopsis thaliana Tsf1* gene encoding elongation factor EF-1α (Axelos et al., 1989)
Intervening Sequence	2661-2669	Sequence used in DNA cloning
TS[5]-*CTP2*	2670-2897	Targeting sequence from the *shkG* gene encoding the chloroplast transit peptide region of *Arabidopsis thaliana* EPSPS (Herrmann, 1995; Klee et al., 1987) that directs transport of the CP4 EPSPS protein to the chloroplast
CS[6]-*cp4 epsps*	2898-4265	Codon optimized coding sequence of the *aroA* gene from the *Agrobacterium* sp. strain CP4 encoding the CP4 EPSPS protein (Barry et al., 2001; Padgette et al., 1996)
Intervening Sequence	4266-4307	Sequence used in DNA cloning
T[7]-*E9*	4308-4950	3' untranslated sequence from the *rbcS2* gene of *Pisum sativum* encoding the Rubisco small subunit (Coruzzi et al., 1984)
Intervening Sequence	4951-4993	Sequence used in DNA cloning
B-Left Border Region [r1]	4994-5267	DNA region from *Agrobacterium tumefaciens* containing the Left Border sequence used for transfer of the T-DNA (Barker et al., 1983; Zambryski et al., 1982)
3' Flanking Sequence	5268-6174	DNA sequence adjacent to the 3' end of the insertion site

[1] B, Border
[2] P, Promoter
[3] L, Leader
[4] I, Intron
[5] TS, Targeting Sequence
[6] CS, Coding Sequence
[7] T, Transcription Termination Sequence
[r1] Superscripts in Left and Right Border Regions indicate that the sequences in MON 88302 were truncated compared to the sequences in PV-BNHT2672.

IV.A. Southern Blot Analysis to Determine Insert and Copy Number of T-DNA I in MON 88302

The numbers of copies and insertion sites of the T-DNA sequences in the canola genome were evaluated by digesting MON 88302 and conventional control genomic DNA samples with the restriction enzyme *Ase* I or the combination of restriction enzymes *Sal* I and *Sca* I and hybridizing Southern blots with probes that span the T-DNA (Figure IV-1). Each restriction digest is expected to produce a specific banding pattern on the Southern blots (Table IV-1). Any additional copies and/or integration sites would be detected as additional bands on the blots.

The restriction enzyme *Ase* I cleaves once within the inserted T-DNA and within the known genomic DNA flanking the 5' and 3' ends of the insert (IV-1). Therefore, if T-DNA sequences were present as a single copy at a single integration site in MON 88302, the digestion with *Ase* I was expected to generate two border segments with expected sizes of ~3.8 kb and ~1.4 kb (Figure IV-1 and Table IV-1). The combination of restriction enzymes *Sal* I and *Sca* I cleaves once within the inserted T-DNA and within the known genomic DNA flanking the 3' end of the insert (Figure IV-1). If T-DNA sequences were present as a single copy at a single integration site in MON 88302, the digestion with *Sal* I and *Sca* I was expected to generate two border segments with expected sizes of >1.8 kb and ~4.3 kb (Figure IV-1 and Table IV-1).

The Southern blots were hybridized with T-DNA probes that collectively span the entire inserted DNA sequence (Figures III-1 and IV-1, Probe 1, Probe 2, and Probe 3). Conventional control genomic DNA digested with the restriction enzyme *Ase* I and spiked with either probe templates and/or digested PV-BNHT2672 DNA served as positive hybridization controls. The positive hybridization control was spiked at approximately 0.1 and 1 genome equivalents to demonstrate sufficient sensitivity of the Southern blot. Conventional control genomic DNA digested with the appropriate restriction enzymes was used as a negative control. The results of these analyses are shown in Figure IV-2 and Figure IV-3.

IV.A.1. T-DNA Probes 1 and 3

Conventional control genomic DNA digested with *Ase* I (Figure IV-2, Lane 1 and Lane 5) or the combination of restriction enzymes *Sal* I and *Sca* I (Figure IV-2, Lane 3 and Lane 7) and simultaneously hybridized with Probe 1 and Probe 3 (Figures III-1 and IV-1) produced no detectable hybridization bands as expected for the negative control. Conventional control genomic DNA digested with *Ase* I and spiked with the PV-BNHT2672 DNA, previously digested with the combination of restriction enzymes *Bam* HI and *Sca* I (Figure III-1), produced two bands at ~7.2 kb and ~2.5 kb (Figure IV-2, Lane 10), as expected. Conventional control genomic DNA digested with *Ase* I and spiked with probe templates of Probe 1 and Probe 3 (Figure III-1) produced the expected bands at ~2.3 kb and ~1.3 kb (Figure IV-2, Lane 11 and Lane 12). Detection of the positive controls indicates that the probes hybridized to their target sequences.

MON 88302 DNA digested with *Ase* I and simultaneously hybridized with Probe 1 and Probe 3 (Figures III-1 and IV-1) produced the expected bands at ~3.8 kb and ~1.4 kb (Figure IV-2, Lane 2 and Lane 6). MON 88302 DNA digested with the combination of restriction enzymes *Sal* I and *Sca* I and hybridized with Probe 1 and Probe 3 (Figures III-1 and IV-1) produced two bands at ~2.7 kb and ~4.3 kb (Figure IV-2, Lane 4 and Lane 8), which is consistent with the expected >1.8 kb and ~4.3 kb bands (Figure IV-1 and Table IV-1), respectively.

The results presented in Figure IV-2 indicate that the sequences covered by Probe 1 and Probe 3 reside at a single detectable locus of integration in MON 88302.

IV.A.2. T-DNA Probe 2

Conventional control DNA digested with *Ase* I (Figure IV-3, Lane 1 and Lane 5) or the combination of restriction enzymes *Sal* I and *Sca* I (Figure IV-3, Lane 3 and Lane 7) and hybridized with Probe 2 (Figures III-1 and IV-1) produced no detectable hybridization bands as expected for the negative control. Conventional control genomic DNA digested with *Ase* I and spiked with the PV-BNHT2672 DNA, previously digested with the combination of restriction enzymes *Bam* HI and *Sca* I (Figure III-1), produced a unique band at ~2.5 kb (Figure IV-3, Lane 10 and Lane 11), as expected. Detection of the positive controls indicates that the probe hybridized to its target sequence.

MON 88302 DNA digested with *Ase* I and hybridized with Probe 2 (Figures III-1 and IV-1) produced the expected band at ~3.8 kb (Figure IV-3, Lane 2 and Lane 6). MON 88302 DNA digested with the combination of restriction enzymes *Sal* I and *Sca* I and hybridized with Probe 2 (Figures III-1 and IV-1) produced the expected band at ~4.3 kb (Figure IV-3, Lane 4 and Lane 8, Figure IV-1, and Table IV-1).

The results presented in Figure IV-3 indicate that the sequence covered by Probe 2 resides at a single detectable locus of integration in MON 88302.

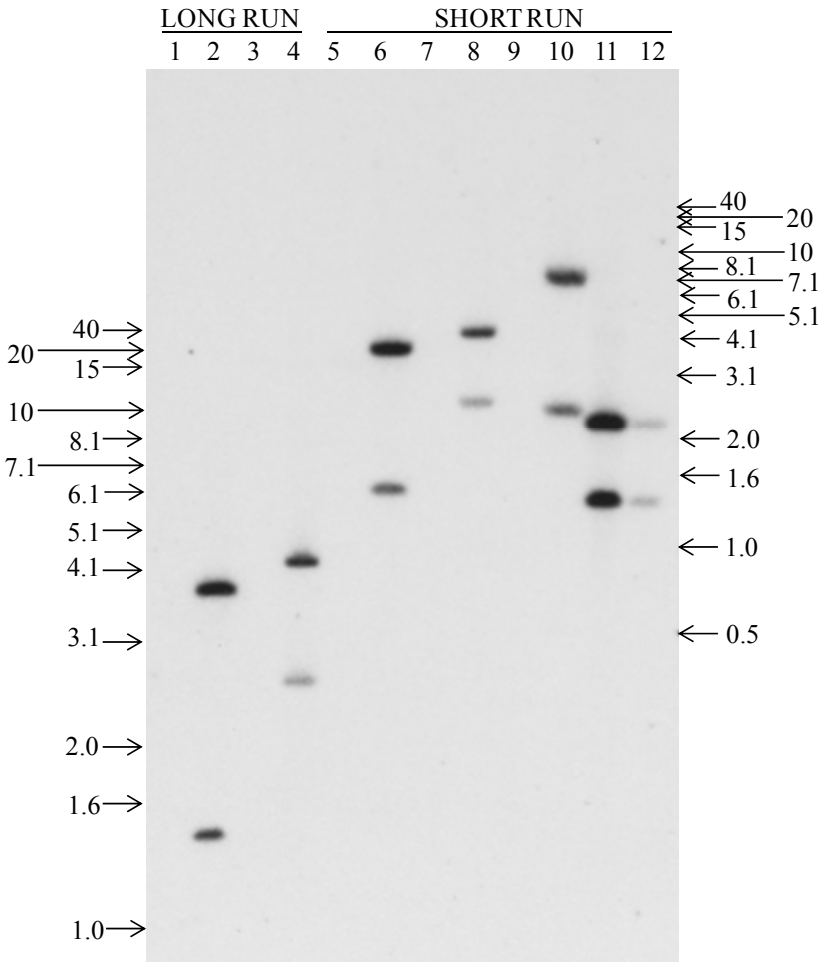


Figure IV-2. Southern Blot Analysis to Determine Insert and Copy Number of T-DNA I in MON 88302: Probes 1 and 3

The blot was simultaneously hybridized with two ^{32}P-labeled probes that span a portion of the T-DNA sequence (Figure III-1, Probe 1 and Probe 3). Each lane contains approximately 10 µg of digested genomic DNA. Arrows denote the size of the DNA, in kilobase pairs, obtained from 1 Kb DNA Extension Ladder on the ethidium bromide stained gel. Lane designations are as follows:

Lane	
1	Conventional control (*Ase* I)
2	MON 88302 (*Ase* I)
3	Conventional control (*Sal* I/*Sca* I)
4	MON 88302 (*Sal* I/*Sca* I)
5	Conventional control (*Ase* I)
6	MON 88302 (*Ase* I)
7	Conventional control (*Sal* I/*Sca* I)
8	MON 88302 (*Sal* I/*Sca* I)
9	Blank
10	Conventional control (*Ase* I) spiked with PV-BNHT2672 (*Bam* HI/*Sca* I) [~1 genome equivalent]
11	Conventional control (*Ase* I) spiked with Probe 1 and Probe 3 [~1 genome equivalent]
12	Conventional control (*Ase* I) spiked with Probe 1 and Probe 3 [~0.1 genome equivalent]

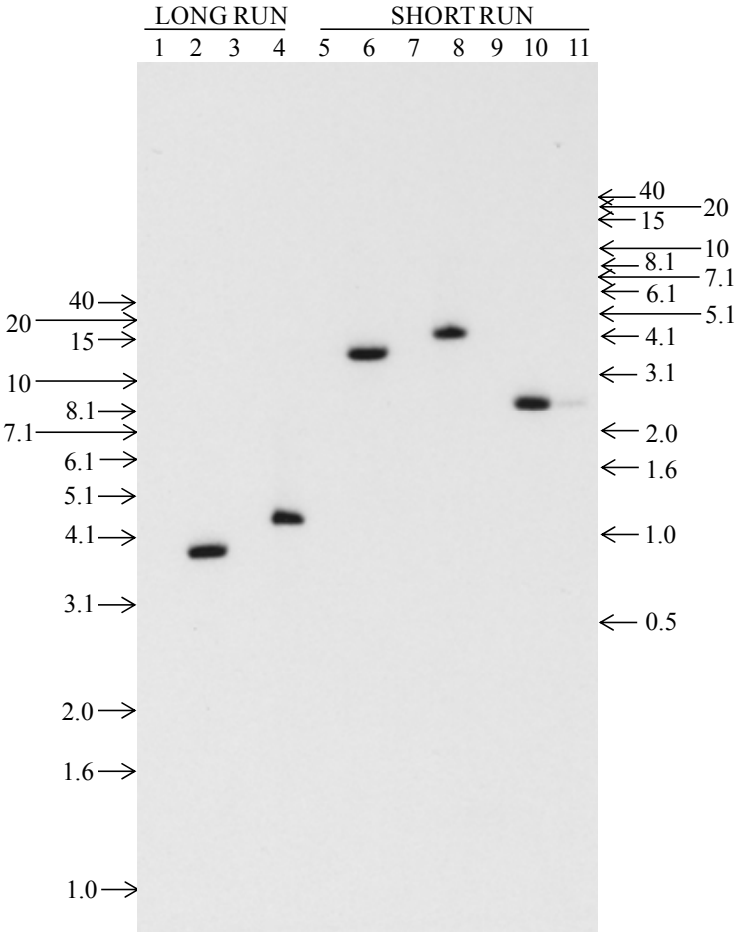


Figure IV-3. Southern Blot Analysis to Determine Insert and Copy Number of T-DNA in MON 88302: Probe 2

The blot was hybridized with a ^{32}P-labeled probe that spans a portion of the T-DNA sequence (Figure III-1, Probe 2). Each lane contains approximately 10 µg of digested genomic DNA. Arrows denote the size of the DNA, in kilobase pairs, obtained from 1 Kb DNA Extension Ladder on the ethidium bromide stained gel. Lane designations are as follows:

Lane	
1	Conventional control (*Ase* I)
2	MON 88302 (*Ase* I)
3	Conventional control (*Sal* I/*Sca* I)
4	MON 88302 (*Sal* I/*Sca* I)
5	Conventional control (*Ase* I)
6	MON 88302 (*Ase* I)
7	Conventional control (*Sal* I/*Sca* I)
8	MON 88302 (*Sal* I/*Sca* I)
9	Blank
10	Conventional control (*Ase* I) spiked with PV-BNHT2672 (*Bam* HI/*Sca* I) [~1 genome equivalent]
11	Conventional control (*Ase* I) spiked with PV-BNHT2672 (*Bam* HI/*Sca* I) [~0.1 genome equivalent]

IV.B. Southern Blot Analysis to Determine the Presence or Absence of PV-BNHT2672 Backbone Sequences in MON 88302

To determine the presence or absence of the PV-BNHT2672 backbone sequences, MON 88302 and conventional control genomic DNA were digested with the restriction enzyme *Ase* I or the combination of restriction enzymes *Sal* I and *Sca* I, and hybridized with one of the three backbone probes that collectively span the entire backbone sequences (Figure III-1, Probe 4, Probe 5, and Probe 6). If backbone sequences are present in MON 88302, then probing with backbone probes should result in hybridizing bands. Conventional control genomic DNA digested with the restriction enzyme *Ase* I and spiked with digested PV-BNHT2672 DNA served as positive hybridization controls. The positive hybridization control was spiked at approximately 0.1 and 1 genome equivalents to demonstrate sufficient sensitivity of the Southern blot. Conventional control genomic DNA digested with the appropriate restriction enzymes was used as a negative control. The results of these analyses are shown in Figures IV-4, IV-5, and IV-6.

IV.B.1. Backbone Probe 4

Conventional control DNA digested with *Ase* I (Figure IV-4, Lane 1 and Lane 5) or the combination of restriction enzymes *Sal* I and *Sca* I (Figure IV-4, Lane 3 and Lane 7) and hybridized with Probe 4 (Figure III-1) produced no detectable hybridization bands as expected for the negative control. Conventional control DNA digested with *Ase* I and spiked with the PV-BNHT2672 DNA, previously digested with the combination of restriction enzymes *Bam* HI and *Sca* I (Figure III-1), produced a unique band at ~7.2 kb (Figure IV-4, Lane 10 and Lane 11), as expected. Detection of the positive controls indicates that the probe hybridized to its target sequence.

MON 88302 DNA digested with *Ase* I (Figure IV-4, Lane 2 and Lane 6) or the combination of restriction enzymes *Sal* I and *Sca* I (Figure IV-4, Lane 4 and Lane 8) and hybridized with Probe 4 produced no detectable bands.

The results presented in Figure IV-4 indicate that MON 88302 contains no detectable backbone sequences covered by Probe 4.

IV.B.2. Backbone Probe 5

Conventional control DNA digested with *Ase* I (Figure IV-5, Lane 1 and Lane 5) or the combination of restriction enzymes *Sal* I and *Sca* I (Figure IV-5, Lane 3 and Lane 7) and hybridized with Probe 5 (Figure III-1) produced no detectable hybridization bands as expected for the negative control. Conventional control DNA digested with *Ase* I and spiked with the PV-BNHT2672 DNA, previously digested with the combination of restriction enzymes *Bam* HI and *Sca* I (Figure III-1), produced a unique band at ~7.2 kb (Figure IV-5, Lane 10 and Lane 11), as expected. Detection of the positive controls indicates that the probe hybridized to its target sequence.

MON 88302 DNA digested with *Ase* I (Figure IV-5, Lane 2 and Lane 6) or the combination of restriction enzymes *Sal* I and Sca I (Figure IV-5, Lane 4 and Lane 8) and hybridized with Probe 5 produced no detectable bands.

The results presented in Figure IV-5 indicate that MON 88302 contains no detectable backbone sequences covered by Probe 5.

IV.B.3. Backbone Probe 6

Conventional control DNA digested with *Ase* I (Figure IV-6, Lane 1 and Lane 5) or the combination of restriction enzymes *Sal* I and *Sca* I (Figure IV-6, Lane 3 and Lane 7) and hybridized with Probe 6 (Figure III-1) produced no detectable hybridization bands as expected for the negative control. Conventional control DNA digested with *Ase* I and spiked with the PV-BNHT2672 DNA, previously digested with the combination of restriction enzymes *Bam* HI and *Sca* I (Figure III-1), produced a unique band at ~7.2 kb (Figure IV-6, Lane 10 and Lane 11), as expected. Detection of the positive controls indicates that the probe hybridized to its target sequence.

MON 88302 DNA digested with *Ase* I (Figure IV-6, Lane 2 and Lane 6) or the combination of restriction enzymes *Sal* I and *Sca* I (Figure IV-6, Lane 4 and Lane 8) and hybridized with Probe 6 produced no detectable bands.

The results presented in Figure IV-6 indicate that MON 88302 contains no detectable backbone sequences covered by Probe 6.

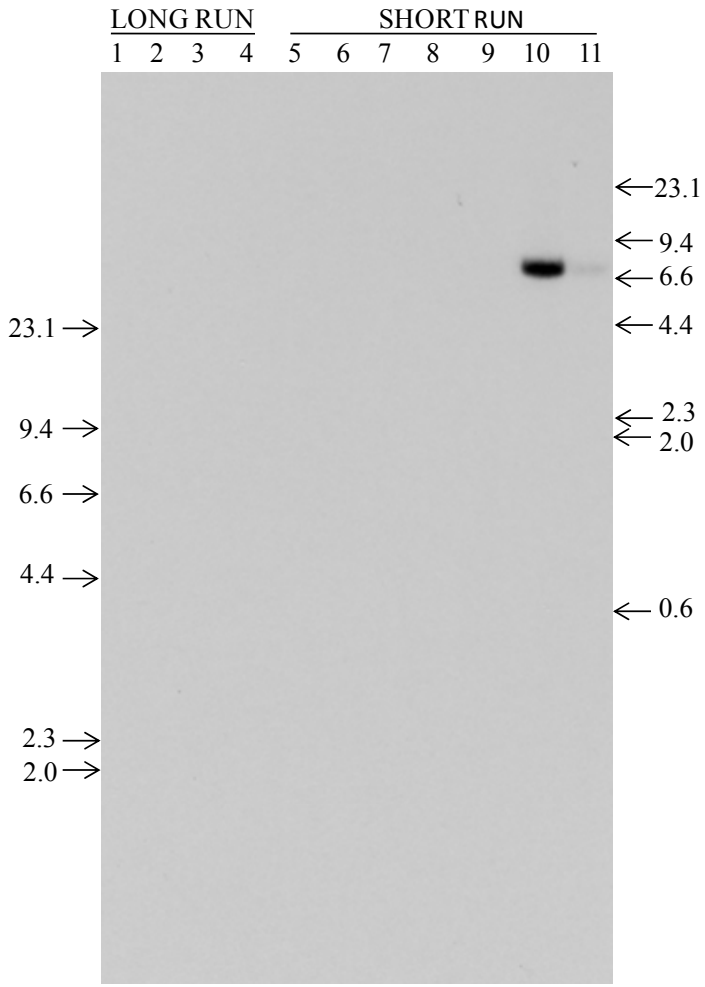


Figure IV-4. Southern Blot Analysis to Determine the Presence or Absence of the PV-BNHT2672 Backbone Sequences in MON 88302: Probe 4

The blot was hybridized with a ^{32}P-labeled probe that spans a portion of the plasmid vector backbone sequences (Figure III-1, Probe 4). Each lane contains approximately 10 µg of digested genomic DNA. Arrows denote the size of the DNA, in kilobase pairs, obtained from λ DNA/*Hind* III fragments on the ethidium bromide stained gel. Lane designations are as follows:

Lane	
1	Conventional control (*Ase* I)
2	MON 88302 (*Ase* I)
3	Conventional control (*Sal* I/*Sca* I)
4	MON 88302 (*Sal* I/*Sca* I)
5	Conventional control (*Ase* I)
6	MON 88302 (*Ase* I)
7	Conventional control (*Sal* I/*Sca* I)
8	MON 88302 (*Sal* I/*Sca* I)
9	Blank
10	Conventional control (*Ase* I) spiked with PV-BNHT2672 (*Bam* HI/*Sca* I) [~1 genome equivalent]
11	Conventional control (*Ase* I) spiked with PV-BNHT2672 (*Bam* HI/*Sca* I) [~0.1 genome equivalent]

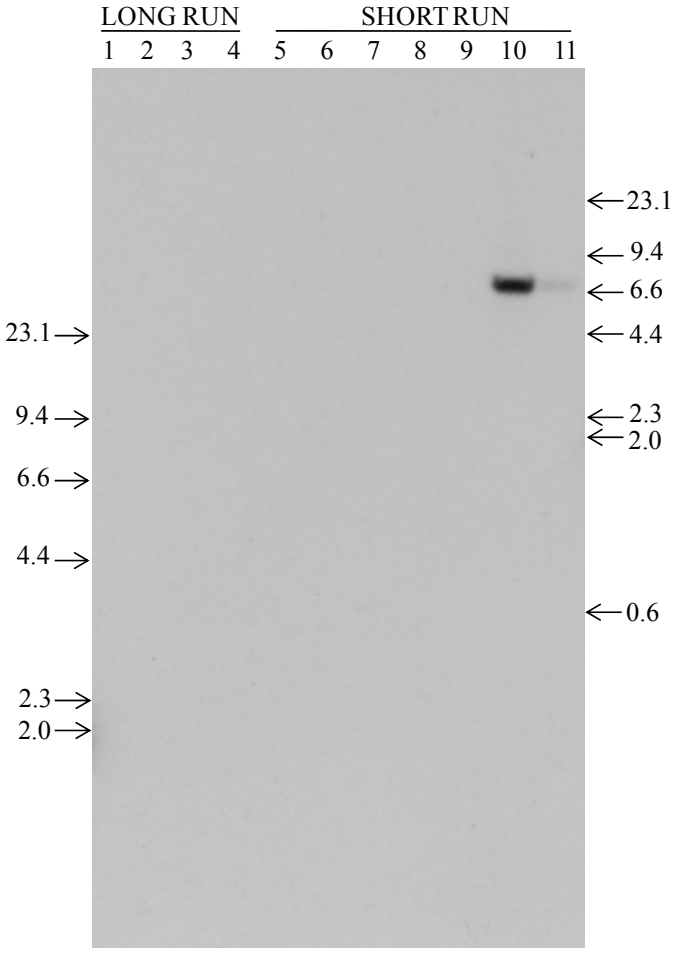


Figure IV-5. Southern Blot Analysis to Determine the Presence or Absence of the PV-BNHT2672 Backbone Sequences in MON 88302: Probe 5

The blot was hybridized with a ^{32}P-labeled probe that spans a portion of the plasmid vector backbone sequences (Figure III-1, Probe 5). Each lane contains approximately 10 µg of digested genomic DNA. Arrows denote the size of the DNA, in kilobase pairs, obtained from λ DNA/*Hind* III fragments the on the ethidium bromide stained gel. Lane designations are as follows:

Lane	
1	Conventional control (*Ase* I)
2	MON 88302 (*Ase* I)
3	Conventional control (*Sal* I/*Sca* I)
4	MON 88302 (*Sal* I/*Sca* I)
5	Conventional control (*Ase* I)
6	MON 88302 (*Ase* I)
7	Conventional control (*Sal* I/*Sca* I)
8	MON 88302 (*Sal* I/*Sca* I)
9	Blank
10	Conventional control (*Ase* I) spiked with PV-BNHT2672 (*Bam* HI/*Sca* I) [~1 genome equivalent]
11	Conventional control (*Ase* I) spiked with PV-BNHT2672 (*Bam* HI/*Sca* I) [~0.1 genome equivalent]

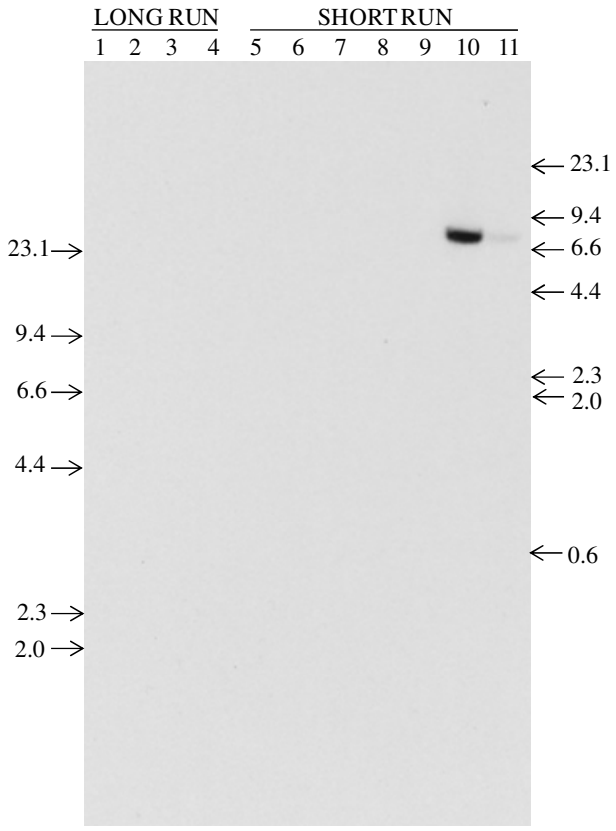


Figure IV-6. Southern Blot Analysis to Determine the Presence or Absence of the PV-BNHT2672 Backbone Sequences in MON 88302: Probe 6

The blot was hybridized with a ^{32}P-labeled probe that spans a portion of the plasmid vector backbone sequences (Figure III-1, Probe 6). Each lane contains approximately 10 µg of digested genomic DNA. Arrows denote the size of the DNA, in kilobase pairs, obtained from λ DNA/*Hind* III fragments on the ethidium bromide stained gel. Lane designations are as follows:

Lane	
1	Conventional control (*Ase* I)
2	MON 88302 (*Ase* I)
3	Conventional control (*Sal* I/*Sca* I)
4	MON 88302 (*Sal* I/*Sca* I)
5	Conventional control (*Ase* I)
6	MON 88302 (*Ase* I)
7	Conventional control (*Sal* I/*Sca* I)
8	MON 88302 (*Sal* I/*Sca* I)
9	Blank
10	Conventional control (*Ase* I) spiked with PV-BNHT2672 (*Bam* HI/*Sca* I) [~1 genome equivalent]
11	Conventional control (*Ase* I) spiked with PV-BNHT2672 (*Bam* HI/*Sca* I) [~0.1 genome equivalent]

IV.C. Organization and Sequence of the Insert and Adjacent DNA in MON 88302

The organization and sequence of the elements within the MON 88302 insert was confirmed by DNA sequence analysis. PCR primers were designed with the intent to amplify two overlapping DNA amplicons that span the entire length of the insert and the associated DNA flanking the 5' and 3' ends of the insert (Figure IV-7). The amplified PCR products were subjected to DNA sequence analyses. This analysis determined that the DNA sequence of the MON 88302 insert is 4428 bp long (Table IV-2) and is identical to the corresponding T-DNA sequence of PV-BNHT2672 as described in Table III-1.

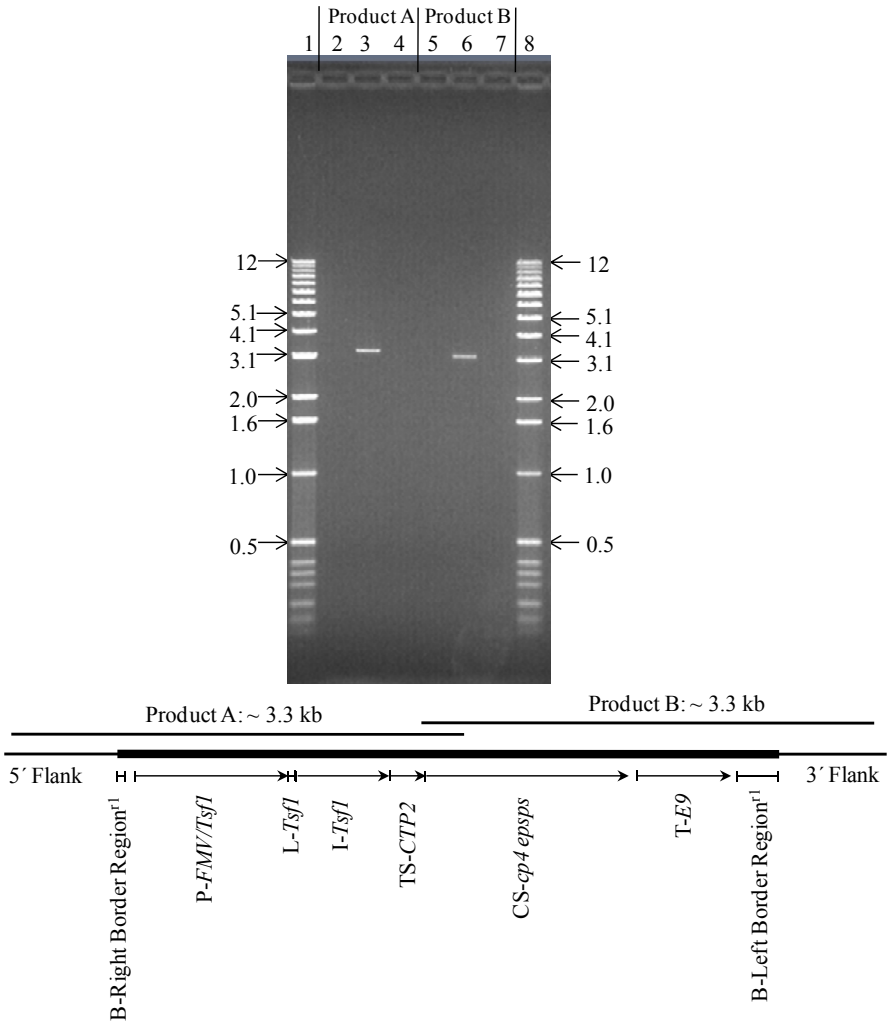


Figure IV-7. Overlapping PCR Analysis across the Insert in MON 88302

PCR was performed on both conventional control genomic DNA and MON 88302 genomic DNA using two pairs of primers to generate overlapping PCR fragments from MON 88302 for sequence analysis. Five microliters of each of the PCR reactions was loaded on the gel. The expected product size for each amplicon is provided in the illustration of the insert in MON 88302 that appears at the bottom of the figure. Arrows on the agarose gel photograph denote the size of the DNA, in kilobase pairs, obtained from 1 Kb DNA Ladder on the ethidium bromide stained gel. Lane designations are as follows:

Lane	
1	1 Kb DNA Ladder
2	Conventional control
3	MON 88302
4	No template DNA control
5	Conventional control
6	MON 88302
7	No template DNA control
8	1 Kb DNA Ladder

IV.D. PCR and DNA Sequence Analyses to Examine the MON 88302 Insertion Site

PCR and sequence analyses were performed on genomic DNA extracted from MON 88302 and the conventional control to examine the MON 88302 insertion site. The PCR was performed with a forward primer specific to the genomic DNA sequence flanking the 5' end of the insert paired with a reverse primer specific to the genomic DNA sequence flanking the 3' end of the insert (Figure IV-8). The amplified PCR product from the conventional control was subjected to DNA sequence analysis. Alignments between the conventional control sequence obtained from this analysis and the sequences immediately flanking the 5' and 3' end of the MON 88302 insert were separately performed to determine the integrity and genomic organization of the insertion site in MON 88302. From these alignment analyses, a 9 base pair insertion immediately adjacent to the 3' end of the MON 88302 insert and a 29 base pair deletion from the conventional genomic DNA were identified. Such changes are quite common during plant transformation; these changes presumably resulted from double-stranded break repair mechanisms in the plant during the *Agrobacterium*-mediated transformation process (Salomon and Puchta, 1998). A single nucleotide difference between the conventional control sequence and the genomic DNA sequence flanking the 3' end of the MON 88302 insert was also identified. The difference was most likely caused by a single nucleotide polymorphism (SNP) segregating in the canola population (Trick et al., 2009).

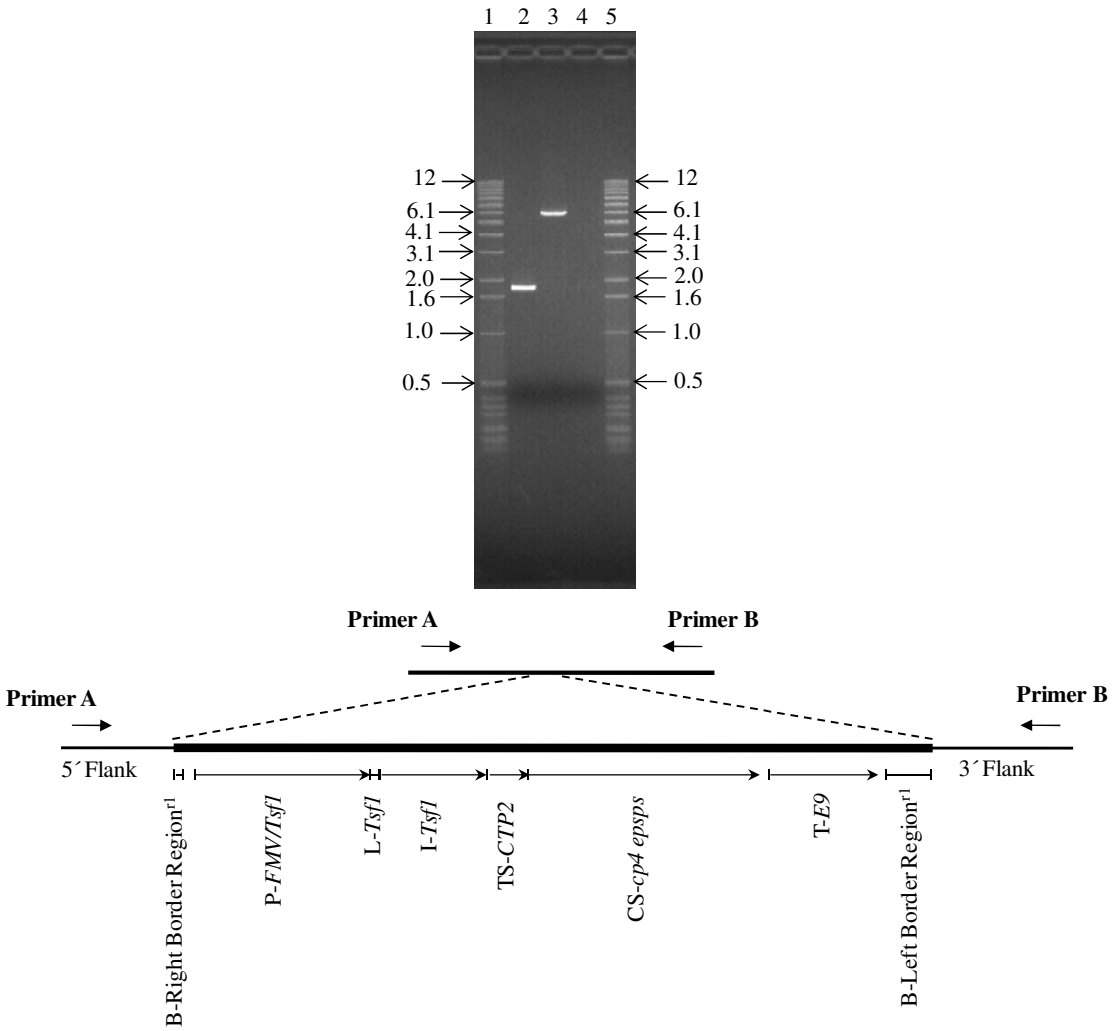


Figure IV-8. PCR Amplification of the MON 88302 Insertion Site in Conventional Canola

PCR was performed on both conventional control genomic DNA and MON 88302 genomic DNA, using Primer A specific to the 5' flanking sequence and Primer B specific to the 3' flanking sequence of the insert in MON 88302, to generate DNA fragments for sequence analysis. The insertion site in conventional control (top) and MON 88302 (bottom) are illustrated at the bottom of the figure. Five microliters of each of the PCR reactions were loaded on the gel. Arrows on the agarose gel photograph denote the size of the DNA, in kilobase pairs, obtained from 1Kb DNA Ladder on the ethidium bromide stained gel. Lane designations are as follows:

Lane	
1	1 Kb DNA Ladder
2	Conventional control
3	MON 88302
4	No template DNA control
5	1 Kb DNA Ladder

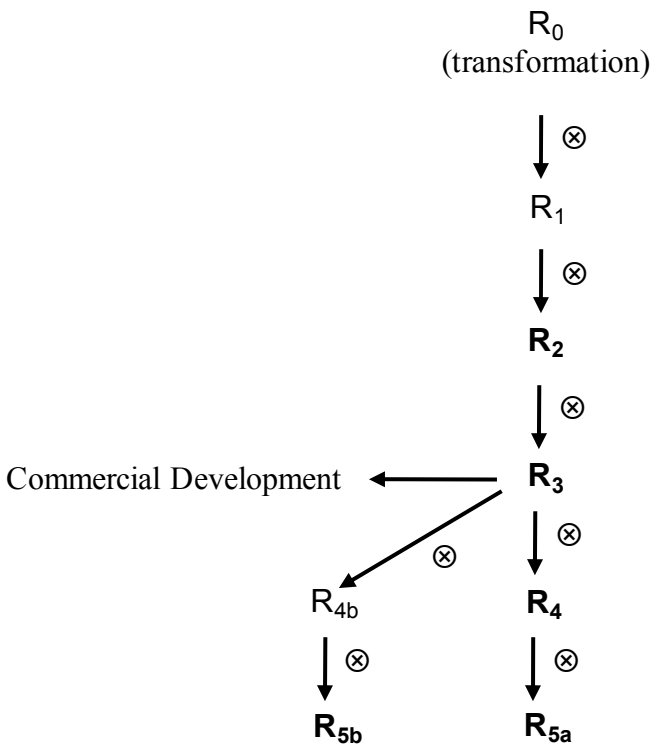
IV.E. Southern Blot Analysis to Examine Insert Stability in Multiple Generations of MON 88302

In order to demonstrate the stability of the insert in MON 88302, Southern blot analysis was performed using genomic DNA extracted from leaf tissues from four breeding generations of MON 88302. For reference, the breeding history of MON 88302 is presented in Figure IV-9. The specific generations tested are indicated in the legend of Figure IV-9. The R_3 generation was used for the molecular characterization analyses shown in Figure IV-2 through Figure IV-6. To analyze insert stability, additional samples from three generations of MON 88302 were evaluated by Southern blot analysis and compared to the R_3 generation. Genomic DNA, isolated from each of the selected generations of MON 88302, was digested with the restriction enzyme *Ase* I and simultaneously hybridized with Probe 1 and Probe 3 (Figures III-1 and IV-1), which was designed to detect both fragments generated by the *Ase* I digest. Any instability associated with the insert would be detected as extra bands within the fingerprint on the Southern blot. The Southern blot has the same controls as described in Section IV.A.1.

IV.E.1. T-DNA Probes 1 and 3

Conventional control genomic DNA digested with restriction enzyme *Ase* I and simultaneously hybridized with Probe 1 and Probe 3 (Figures III-1 and IV-1) produced no hybridization signals (Figure IV-10, Lane 1) as expected for the negative control. Conventional control genomic DNA digested with *Ase* I and spiked with the PV-BNHT2672 DNA, previously digested with the combination of restriction enzymes *Bam* HI and *Sca* I (Figure III-1 and Table IV-1), produced the expected bands at ~2.5 kb and ~7.2 kb (Figure IV-10, Lane 8). Conventional control genomic DNA digested with *Ase* I and spiked with probe templates of Probe 1 and Probe 3 produced the expected bands at ~2.3 kb and ~1.3 kb (Figure IV-10, Lane 9 and Lane 10). Detection of the positive controls indicates that the probes hybridized to their target sequences.

MON 88302 genomic DNA digested with *Ase* I and hybridized with Probe 1 and Probe 3 (Figures III-1 and IV-1) is expected to produce a Southern fingerprint with two bands at ~3.8 kb and ~1.4 kb (Figure IV-1 and Table IV-1). Southern fingerprints produced from multiple generations (Figure IV-10, Lane 2, Lane 4, Lane 5, and Lane 6), of MON 88302 are consistent with the one produced from the fully characterized generation R_3 (Figure IV-2, Lane 2 and Lane 6, and Figure IV-10, Lane 3), indicating that MON 88302 contains one copy of the T-DNA insert that is stable across multiple generations.



R_0- Originally transformed plant ; ⊗-self pollination

Figure IV-9. Breeding History of MON 88302
R_0 corresponds to the transformed canola plant. All generations were self pollinated. ⊗ designates self-pollination. The R_3 generation was used for the molecular characterization and commercial development of MON 88302. The R_2, R_3, R_4, R_{5a}, and R_{5b} (bolded in the breeding tree) generations of MON 88302 were used for analyzing the stability of the insert across generations. R_{5b} was propagated independently of R_{5a} beginning with the R_3 generation.

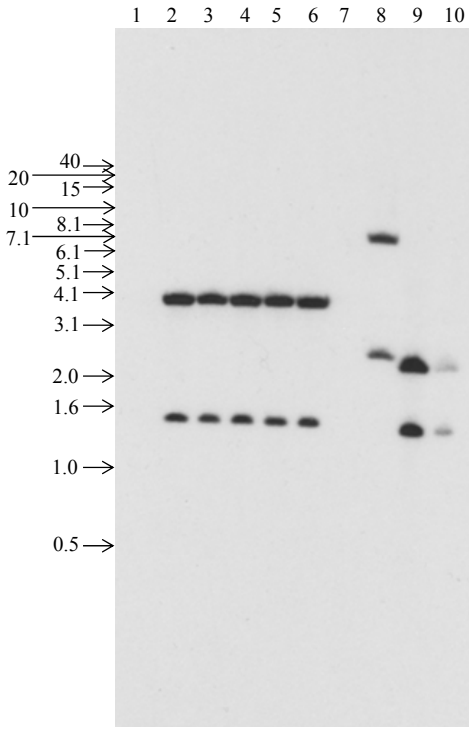


Figure IV-10. Southern Blot Analysis to Examine Insert Stability in Multiple Generations of MON 88302: Probes 1 and 3

The blot was simultaneously hybridized with two ^{32}P-labeled probes that span a portion of the T-DNA sequence (Figure III-1, Probe 1 and Probe 3). Each lane contains ~10 µg of digested genomic DNA. Arrows denote the size of the DNA, in kilobase pairs, obtained from 1Kb DNA Extension Ladder on the ethidium bromide stained gel. Lane designations are as follows:

Lane	
1	Conventional control (*Ase* I)
2	R_2 generation of MON 88302 (*Ase* I)
3	R_3 generation of MON 88302 (*Ase* I)
4	R_4 generation of MON 88302 (*Ase* I)
5	R_{5a} generation of MON 88302 (*Ase* I)
6	R_{5b} generation of MON 88302 (*Ase* I)
7	Blank
8	Conventional control (*Ase* I) spiked with PV-BNHT2672 (*Bam* HI/*Sca* I) [~1 genome equivalent]
9	Conventional control (*Ase* I) spiked with probe templates Probe 1 and Probe 3 [~1 genome equivalent]
10	Conventional control (*Ase* I) spiked with probe templates Probe 1 and Probe 3 [~0.1 genome equivalent]

IV.F. Inheritance of the Genetic Insert in MON 88302

During development of MON 88302, segregation data were recorded to assess the inheritance and stability of the coding sequence present in MON 88302. Chi-square (χ^2) analysis was performed over several generations to confirm the segregation and stability of the MON 88302 insert. The χ^2 analysis is based on testing the observed segregation ratio to the expected segregation ratio according to Mendelian principles.

The MON 88302 breeding path for generating segregation data is described in Figure IV-11. The transformed R_0 plant was self-pollinated to generate R_1 seed. From the R_1 segregating population, an individual plant homozygous for the *cp4 epsps* coding sequence (subsequently designated MON 88302) was identified via TaqMan PCR copy number assay and Southern blot copy number analysis. The *cp4 epsps* homozygous R_1 plant was self-pollinated to give rise to R_2 plants that were self-pollinated to produce R_3 seed. At each generation, the homozygous plants were tested for the expected segregation pattern of 1:0 (positive: negative) for the *cp4 epsps* gene using a glyphosate spray test and/or TaqMan PCR assay.

An individual *cp4 epsps* positive R_3 plant, which was confirmed by Endpoint TaqMan PCR assay, was crossed to a Monsanto proprietary canola inbred, which does not contain the MON 88302 insert, via traditional breeding techniques to produce hemizygous F_1 seed. The resulting F_1 plant, was shown to contain a single copy of the *cp4 epsps* gene by real-time TaqMan PCR, and was then self-pollinated to produce F_2 seed. A *cp4 epsps* hemizygous F_2 plant from the F_2 population was shown to contain a single copy of the *cp4 epsps* gene by real-time TaqMan PCR and was then self-pollinated to produce the F_3 population. A *cp4 epsps* hemizygous F_3 plant from the F_3 population was shown to contain a single copy of the *cp4 epsps* gene by real-time TaqMan PCR and was self-pollinated to produce the F_4 population. The copy number of the *cp4 epsps* gene in the F_2, F_3, and F_4 populations was then assessed using a real-time TaqMan PCR assay.

A χ^2 analysis was performed on each of the F_2, F_3, and F_4 populations using the statistical program R (Version 2.10.1) to compare the observed segregation ratio of *cp4 epsps* coding sequence to the expected ratio according to Mendelian principles of inheritance. The Chi-square was calculated as:

$$\chi^2 = \sum [(o - e)^2 / e]$$

where o = observed frequency of the genotype or phenotype and e = expected frequency of the genotype or phenotype. The level of statistical significance was predetermined to be 5% ($\alpha = 0.05$).

The results of the χ^2 analysis of the MON 88302 segregating progeny are presented in Table IV-3. The χ^2 value in the F_2, F_3, and F_4 populations indicated no statistically significant difference between the observed and expected 1:2:1 segregation ratio

(homozygous positive: hemizygous: homozygous negative) of *cp4 epsps* coding sequence. These results support the conclusion that the *cp4 epsps* expression cassette in MON 88302 resides at a single locus within the canola genome and is inherited according to Mendelian principles of inheritance. These results are also consistent with the molecular characterization data indicating that MON 88302 contains a single, intact copy of the *cp4 epsps* expression cassette inserted at a single locus in the canola genome.

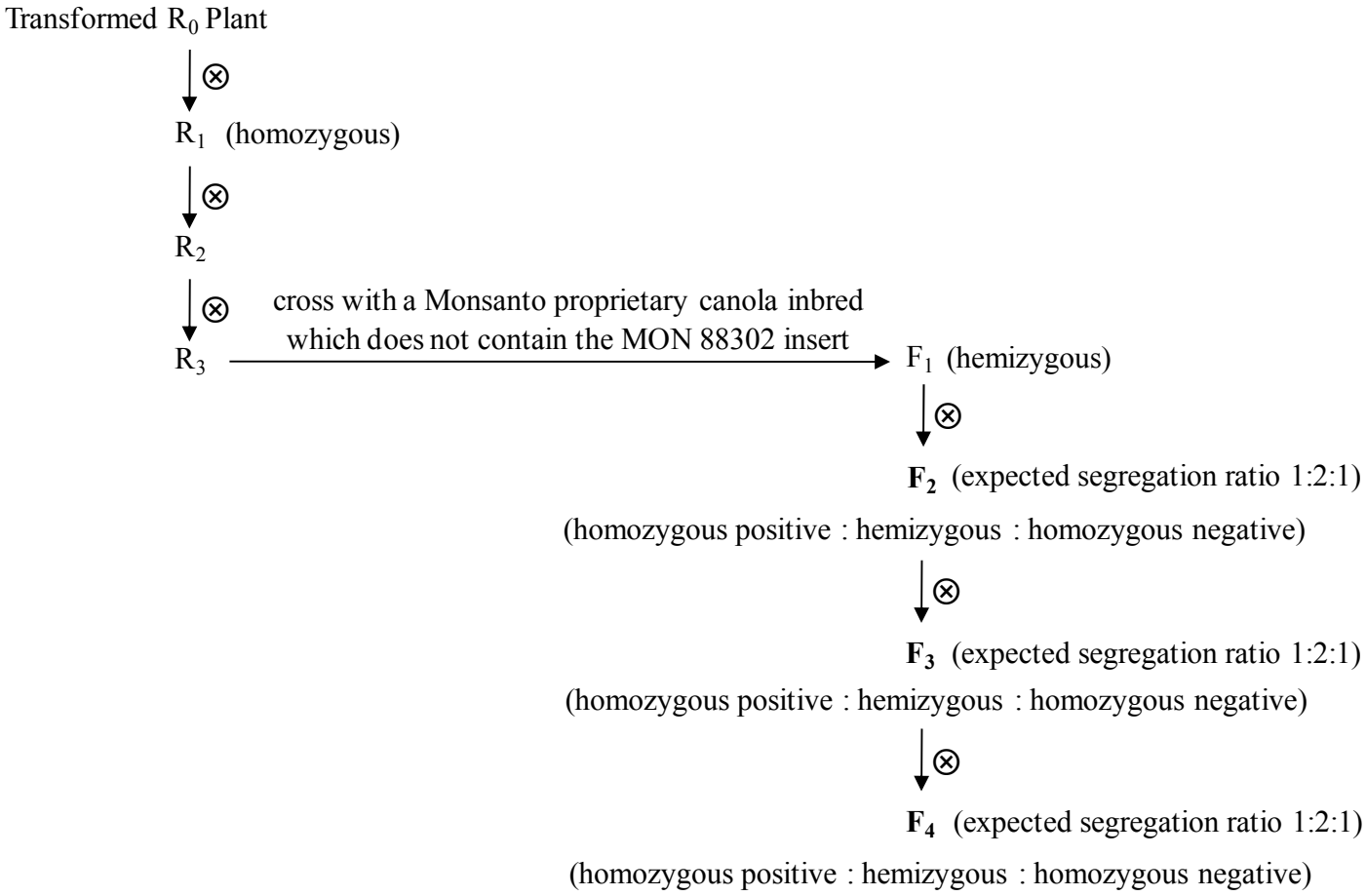


Figure IV-11. Breeding Path for Generating Segregation Data for MON 88302
An individual hemizygous plant from each of the F_1, F_2, and F_3 populations was self-pollinated to produce the population of the next generation. Chi-square analyses were conducted on segregation data from the F_2, F_3, and F_4 populations.

Table IV-3. Segregation of the *cp4 epsps* Gene During the Development of MON 88302

					1:2:1 Segregation				
Generation	**Total Plants***	**Observed # Plants Homozygous Positive**	**Observed # Plants Hemizygous**	**Observed # Plants Homozygous Negative**	**Expected # Plants Homozygous Positive**	**Expected # Plants Hemizygous**	**Expected # Plants Homozygous Negative**	**χ^2**	**Probability**
F_2	220	51	122	47	55.00	110.00	55.00	2.76	0.2511
F_3	166	39	94	33	41.50	83.00	41.50	3.35	0.1874
F_4	198	53	97	48	49.50	99.00	49.50	0.33	0.8465

*Plants were evaluated for the copy number of the *cp4 epsps* gene using a real-time TaqMan PCR assay.

IV.G. Genetic Modification Characterization Conclusion

Molecular characterization of MON 88302 by Southern blot analyses demonstrated that the T-DNA was inserted into the canola genome at a single locus containing one copy of the *cp4 epsps* expression cassette. No additional elements were detected other than those associated with the insert. Moreover, no backbone sequence was detected in the genome of MON 88302.

DNA sequence analyses performed on MON 88302 determined the complete DNA sequence of the insert in MON 88302, confirmed the predicted organization of the genetic elements within the insert, determined the sequences flanking the insert, and examined the MON 88302 insertion site. Sequence analysis of the T-DNA insertion site indicated that a 9 base pair insertion immediately adjacent to the 3' end of the MON 88302 insert and a 29 base pair deletion from the conventional genomic DNA occurred during the insertion of the T-DNA into the conventional canola to form MON 88302. In addition, a single nucleotide difference between the conventional control sequence and the known DNA sequence flanking the 3' end of the MON 88302 insert was also identified. This single nucleotide difference was most likely caused by single nucleotide polymorphism (SNP) segregating in the canola population (Trick et al., 2009).

Southern blot analysis of multiple MON 88302 generations demonstrated that the inserted DNA has been stably maintained through multiple generations of breeding, thereby, confirming the stability of the insert. Results from segregation analyses show inheritance and stability of the insert were as expected across multiple generations, which corroborates the molecular insert stability analysis and establishes the genetic behavior of the T-DNA in MON 88302 at a single chromosomal locus.

V. CHARACTERIZATION AND SAFETY ASSESSMENT OF THE CP4 EPSPS PROTEIN PRODUCED IN MON 88302

Characterization of the introduced protein in a biotechnology-derived crop is important to establishing food, feed, and environmental safety. As described in Section IV, MON 88302 contains a *cp4 epsps* expression cassette that, when transcribed and translated, results in the expression of the CP4 EPSPS protein.

This section summarizes: 1) the identity and function of the CP4 EPSPS protein produced in MON 88302; 2) demonstration of the equivalence of the plant-produced and *E. coli*-produced proteins; 3) the level of the CP4 EPSPS protein in MON 88302 plant tissues; 4) assessment of the potential allergenicity of the CP4 EPSPS protein produced in MON 88302; and 5) the food and feed safety assessment of the CP4 EPSPS protein produced in MON 88302. The data support a conclusion that MON 88302 is safe for the human or animal consumption based on several lines of evidence summarized below.

V.A. Identity and Function of the CP4 EPSPS Protein from MON 88302

The enzyme, 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), catalyzes one of the enzymatic steps of the shikimic acid pathway, and is the target for the broad spectrum herbicide glyphosate (Haslam, 1993; Herrmann and Weaver, 1999; Kishore et al., 1988; Steinrücken and Amrhein, 1980). The shikimic acid pathway and EPSPS enzymes are ubiquitous to plants and microorganisms, but absent in mammals, fish, birds, reptiles, and insects (Alibhai and Stallings, 2001). EPSPS proteins have been isolated from both plant and microbial sources and their properties have been extensively studied (Harrison et al., 1996; Haslam, 1993; Klee et al., 1987; Schönbrunn et al., 2001; Steinrücken and Amrhein, 1984). The plant and microbial enzymes are mono-functional with a molecular weight of 44-51 kDa (Franz et al., 1997; Kishore et al., 1988). EPSPS enzymes catalyze the transfer of the enolpyruvyl group from phosphoenolpyruvate (PEP) to the 5-hydroxyl of shikimate-3-phosphate (S3P), thereby yielding inorganic phosphate and 5-enolpyruvylshikimate-3-phosphate (EPSP) (Alibhai and Stallings, 2001). Shikimic acid is a substrate for the biosynthesis of the aromatic amino acids (phenylalanine, tryptophan and tyrosine) and other aromatic molecules necessary for plant growth.

The EPSPS transgene in MON 88302 is derived from *Agrobacterium* sp. strain CP4 (*cp4 epsps*). The *cp4 epsps* coding sequence encodes a 47.6 kDa EPSPS protein consisting of a single polypeptide of 455 amino acids (Padgette et al., 1996). The CP4 EPSPS protein is similar and functionally identical to endogenous plant EPSPS enzymes, but has a much reduced affinity for glyphosate, the active ingredient in Roundup agricultural herbicides, relative to endogenous plant EPSPS (Padgette et al., 1996). In conventional plants, glyphosate blocks the biosynthesis of EPSP, thereby depriving plants of essential amino acids (Haslam, 1993; Steinrücken and Amrhein, 1980). In Roundup Ready plants, which are tolerant to Roundup agricultural herbicides, requirements for aromatic amino acids and other metabolites are met by the continued action of the CP4 EPSPS enzyme in the presence of glyphosate (Padgette et al., 1996). The CP4 EPSPS protein expressed in MON 88302 is identical to the CP4 EPSPS proteins in other Roundup Ready crops including Roundup Ready soybeans and Roundup Ready 2 Yield soybeans, as well as the

CP4 EPSPS in Roundup Ready corn 2, Roundup Ready canola, Roundup Ready sugar beet, Roundup Ready and Roundup Ready Flex cotton and Roundup Ready alfalfa.

V.B. Characterization and Equivalence of CP4 EPSPS Protein from MON 88302

The safety assessment of crops derived through biotechnology includes characterization of the physicochemical and functional properties of the protein(s) produced from the inserted DNA, and confirmation of the safety of the protein(s). The safety of *E. coli*-produced CP4 EPSPS protein has been assessed previously and the results are summarized by Harrison et al. (1996). For the existing CP4 EPSPS safety data set to be applied to CP4 EPSPS protein produced in MON 88302, the equivalence of the plant- and *E. coli*-produced protein was established. The MON 88302-produced CP4 EPSPS protein was characterized and the equivalence of the physicochemical characteristics and functional activity between the MON 88302-produced CP4 EPSPS protein and the *E. coli*-produced CP4 EPSPS protein was assessed using a panel of analytical tests, including: 1) N-terminal sequence analysis of the MON 88302-produced CP4 EPSPS protein to establish identity; 2) matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) analysis of peptides derived from tryptic digested MON 88302-produced CP4 EPSPS protein to establish identity; 3) western blot analysis using anti-CP4 EPSPS polyclonal antibodies to establish identity and immunoreactive equivalence between MON 88302-produced protein and the *E. coli*-produced protein; 4) sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) to establish equivalence of the apparent molecular weight between MON 88302-produced protein and the *E. coli*-produced protein; 5) glycosylation analysis of the MON 88302-produced CP4 EPSPS protein to establish the equivalence between the MON 88302-produced and *E. coli*-produced CP4 EPSPS proteins; and 6) CP4 EPSPS enzymatic activity analysis to demonstrate functional equivalence between MON 88302-produced and the *E. coli*-produced protein. The details of the materials, methods, and results are described in Appendix C while the conclusions are summarized as follows.

A comparison of the MON 88302-produced CP4 EPSPS protein to the *E. coli*-produced CP4 EPSPS protein confirmed the identity of the MON 88302-produced CP4 EPSPS protein and established the equivalence of the plant produced protein to the *E. coli*-produced CP4 EPSPS protein. The identity of the CP4 EPSPS protein isolated from the seed of MON 88302 was confirmed by N-terminal sequencing, MALDI-TOF MS analysis of peptides produced after trypsin digestion, and by western blot analysis using anti-CP4 EPSPS polyclonal antibodies. The N-terminus of the MON 88302-produced CP4 EPSPS protein matched the predicted amino acid sequence translated from the *cp4 epsps* coding sequence. The MALDI-TOF MS analysis yielded peptide masses consistent with the expected peptide masses from the translated *cp4 epsps* coding sequence. The MON 88302-produced CP4 EPSPS protein was detected on a western blot probed with antibodies specific for CP4 EPSPS protein. Furthermore, the immunoreactive properties and electrophoretic mobility of the MON 88302-produced CP4 EPSPS protein were shown to be equivalent to those of the *E. coli*-produced CP4 EPSPS protein.. The apparent molecular weight, glycosylation status, and functional activity of the MON 88302-produced CP4 EPSPS protein and *E. coli*-produced CP4 EPSPS protein were also all found to be equivalent. Taken together, these data

provide a detailed characterization of the CP4 EPSPS protein isolated from MON 88302 and establish its equivalence to the *E. coli*-produced CP4 EPSPS protein. Furthermore, since CP4 EPSPS proteins isolated from other Roundup Ready crops have been previously demonstrated to be equivalent to the *E. coli*-produced CP4 EPSPS protein, by inference, the MON 88302-produced CP4 EPSPS protein is equivalent to the CP4 EPSPS proteins expressed in other Roundup Ready crops, all of which have been deregulated by USDA-APHIS.

V.C. Expression Levels of CP4 EPSPS Protein in MON 88302

CP4 EPSPS protein levels in various tissues of MON 88302 relevant to the risk assessment were determined by a validated enzyme-linked immunosorbent assay (ELISA). Tissues of MON 88302 were collected from four replicate plots planted in a randomized complete block field design during the 2009 growing season from the following three field sites in the U.S.: Power County, Idaho; Wilkin County, Minnesota; and McHenry County, North Dakota, and the following three field sites in Canada: Portage la Prairie, Manitoba; Newton, Manitoba; and Saskatoon, Saskatchewan. These field sites were representative of canola producing regions suitable for commercial production. Forage, seed, over-season leaf (OSL-1 through OSL-4), and root (Root-1 and Root-2) tissue samples were collected from each replicated plot at all field sites.

CP4 EPSPS protein levels were determined in all eight tissue types. The results obtained from ELISA are summarized in Table V-1 and the details of the materials and methods are described in Appendix D. CP4 EPSPS protein levels in MON 88302 across tissue types ranged from 22 to 500 µg/g dw. The mean CP4 EPSPS protein levels were determined across six sites with the exception of seed (5 sites), OSL-1 (5 sites), OSL-2 (3 sites), and Root-2 (4 sites). Sample collections are detailed in Appendix D. The mean CP4 EPSPS protein levels were highest in leaf (ranging from OSL-1 at 180 µg/g dw to OSL-3 at 230 µg/g dw), followed by forage (170 µg/g dw), root (ranging from Root-2 at 38 µg/g dw to Root-1 at 82 µg/g dw), and seed (27 µg/g dw).

Table V-1. Summary of CP4 EPSPS Protein Levels in Canola Tissues from MON 88302 Grown in 2009 U.S. and Canadian Field Trials

Tissue[1]	Development Stage[2]	Days After Planting (DAP)	CP4 EPSPS Mean (SD) Range (µg/g fw)[3]	CP4 EPSPS Mean (SD) Range (µg/g dw)[4]	LOQ/LOD[5] (µg/g fw)
Forage	30 BBCH	37 – 57	18 (4.4) 14 - 28	170 (22) 120 - 210	0.91/0.28
Seed	99 BBCH	118 – 132	25 (5.2) 21 - 43	27 (5.6) 22 - 46	0.91/0.81
OSL-1	13-14 BBCH	23 - 40	23 (10) 10 - 45	180 (40) 110 - 250	0.91/0.098
OSL-2	17-19 BBCH	32 - 54	22 (5.9) 18 - 37	180 (41) 120 - 250	0.91/0.098
OSL-3	30 BBCH	37 - 57	31 (6.3) 20 - 41	230 (50) 130 - 300	0.91/0.098
OSL-4	60-62 BBCH	51 - 61	36 (14) 20 - 85	210 (80) 110 - 500	0.91/0.098
Root-1	30 BBCH	37 - 57	19 (4.1) 11 - 25	82 (17) 46 - 100	0.91/0.60
Root-2	71-73 BBCH	49 - 81	10 (3.3) 7.0 - 17	38 (14) 24 - 62	0.91/0.60

[1]OSL = over-season leaf.

[2]The development stage at which each tissue was collected. The canola growth stages are based on the Bayer, BASF, Ciba-Geigy and Hoechst Cereal Grain Growth Scale (BBCH) (BBCH, 2001).

[3]Protein levels are expressed as the arithmetic mean and standard deviation (SD) as microgram (µg) of protein per gram (g) of tissue on a fresh weight basis (fw). The means, SD, and ranges (minimum and maximum values) were calculated for each tissue across all sites. The numbers of samples (n) figured into the calculations are as follows: forage n = 20, seed n = 16, OSL-1 n = 16, OSL-2 n = 9, OSL-3 n = 20, OSL-4 n = 20, Root-1 n = 19, and Root-2 n = 11. Sample collections are detailed in Appendix D.

[4]Protein levels are expressed as the arithmetic mean and standard deviation (SD) as microgram (µg) of protein per gram (g) of tissue on a dry weight basis (dw). The dry weight values were calculated by dividing the µg/g fw by the dry weight conversion factor obtained from moisture analysis data.

[5]LOQ=limit of quantitation; LOD = limit of detection.

V.D. Assessment of Potential Allergenicity of the CP4 EPSPS Protein

The allergenic potential of an introduced protein is assessed by comparing the biochemical characteristics of the introduced protein to biochemical characteristics of known allergens (Codex Alimentarius, 2009). A protein is not likely to be associated with allergenicity if: 1) the protein is from a non-allergenic source; 2) the protein represents a very small portion of the total plant protein; 3) the protein does not share structural similarities to known allergens based on the amino acid sequence; and 4) the protein is rapidly digested in mammalian gastrointestinal systems. The CP4 EPSPS protein has been assessed for its potential allergenicity according to these safety assessment guidelines.

1) The CP4 EPSPS protein originates from *Agrobacterium sp.* strain CP4 an organism that has not been reported to be a source of known allergens.

2) The CP4 EPSPS protein represents no more than 0.01% of the total protein in the seed of MON 88302.

3) Bioinformatics analyses demonstrated that the CP4 EPSPS protein does not share amino acid sequence similarities with known allergens and, therefore, is highly unlikely to contain immunologically cross-reactive allergenic epitopes.

4) Finally, *in vitro* digestive fate experiments conducted with the CP4 EPSPS protein demonstrate that the protein is rapidly digested in simulated gastric fluid (SGF) and in simulated intestinal fluid (SIF).

Taken together, these data support the conclusion that the CP4 EPSPS protein does not pose a significant allergenic risk to humans or animals.

V.E. Safety Assessment Summary of CP4 EPSPS Protein in MON 88302

Numerous factors have been considered in the safety assessment of the CP4 EPSPS protein and a comprehensive food and feed assessment of CP4 EPSPS protein was conducted. The results are summarized below along with the conclusions reached from the assessment.

V.E.1. The Donor Organism Has a History of Safe Use

The donor organism, *Agrobacterium sp.*, strain CP4 is not known for human or animal pathogenicity, and is not commonly allergenic (FAO-WHO, 1991). *Agrobacterium* sp. strain CP4 has been previously reviewed as a part of the safety assessment of the donor organism during Monsanto consultations with the FDA regarding Roundup Ready soybean (1994), Roundup Ready canola (1995), Roundup Ready cotton (1995), Roundup Ready 2 corn (1996), Roundup Ready sugar beet (1998), and Roundup Ready Flex cotton (2005). Further, the Environmental Protection Agency has established an exemption from the requirement of a tolerance for residues of CP4 EPSPS protein and the genetic material necessary for its production in all plants (U.S. EPA, 1996b).

V.E.2. CP4 EPSPS Proteins Are Common in Food and Feeds

The CP4 EPSPS protein present in MON 88302 is similar to EPSPS proteins consumed in a variety of food and feed sources. CP4 EPSPS protein is homologous to EPSPS proteins naturally present in plants, including food crops (*e.g.,* soybean and maize) and fungal and microbial food sources such as baker's yeast (*Saccharomyces cerevisiae*), all of which have a history of safe human consumption (Harrison et al., 1996; Padgette et al., 1996). The similarity of the CP4 EPSPS protein to EPSPS proteins in a variety of foods supports extensive human consumption of the family of EPSPS proteins and the lack of health concerns. The ubiquitous presence of homologous EPSPS enzymes in food crops and common microorganisms establishes that EPSPS proteins, and their enzymatic activity, pose no hazards for human and animal consumption.

V.E.3. CP4 EPSPS Catalyzes a Specific Enzyme Reaction

EPSPS exerts its functions in the shikimate pathway that is integral to aromatic amino acid biosynthesis in plants and microorganisms (Levin and Sprinson, 1964; Steinrücken and Amrhein, 1980). Therefore, this enzyme and its activity are found widely in food and feed derived from plant and microbial sources. Genes for numerous EPSPS proteins have been cloned (Padgette et al., 1996) and the catalytic domains of this group of proteins are conserved. Bacterial EPSPS proteins have been well characterized with respect to their three dimensional X-ray crystal structures (Stallings et al., 1991) and detailed kinetic and chemical mechanisms (Anderson and Johnson, 1990).

V.E.4. CP4 EPSPS Protein in MON 88302 is Not Homologous to Known Allergens or Toxins

The CP4 EPSPS protein does not share amino acid sequence similarities with known allergens or protein toxins that have adverse effects to mammals. This has been demonstrated by extensive assessment with bioinformatic tools, such as the FASTA sequence alignment tool and eight-amino acid sliding window search. An amino acid sequence is considered to have allergenic potential if it has an exact sequence identity of at least eight linearly contiguous amino acids with a potential allergen epitope (Hileman et al., 2002; Metcalfe et al., 1996). Using a sliding window of less than eight amino acids can produce matches containing significant uncertainty depending on the length of the query sequence (Silvanovich et al., 2006) and are not useful to the allergy assessment process (Thomas et al., 2005).

V.E.5. CP4 EPSPS Protein in MON 88302 Is Labile in *in vitro* Digestion Assays

The CP4 EPSPS protein is readily digestible in simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) (Harrison et al., 1996). Rapid degradation of the full-length CP4 EPSPS protein in SGF and SIF reduces the exposure of CP4 EPSPS protein to cells of the small intestine in a biologically active form.

V.E.6. CP4 EPSPS Protein in MON 88302 is Not Acutely Toxic

An acute oral toxicology study with mice was conducted with a CP4 EPSPS protein (Harrison et al., 1996) that was shown to be physicochemically and functionally equivalent to the CP4 EPSPS protein produced in MON 88302. Results indicate that the CP4 EPSPS protein did not cause any adverse effects in mice, therefore the No Observable Adverse Effect Level (NOAEL) for CP4 EPSPS is considered to be 572 mg/kg, the highest dose level tested.

V.E.6.1 Human and Animal Exposure to the CP4 EPSPS Protein

A common approach used to assess potential health risks from chemicals or other potentially toxic products is to calculate a Margin of Exposure (MOE) between the lowest NOAEL from an appropriate animal toxicity study and an estimate of human exposure. However, the primary human food currently produced from canola is refined, bleached, and deodorized (RBD) oil. Because RBD oil contains negligible amounts of protein (Martín-Hernández et al., 2008), oil produced from MON 88302 will contain negligible levels of CP4 EPSPS protein. Therefore an MOE was not calculated for the CP4 EPSPS protein since there is minimal, if any, dietary exposure to this protein from consumption of foods derived from MON 88302. Furthermore, the safety of CP4 EPSPS has been extensively assessed (Harrison et al., 1996) and several Roundup Ready crops that produce CP4 EPSPS have been reviewed by FDA and other regulatory agencies. They concluded the Roundup Ready crops were safe for consumption.

The potential CP4 EPSPS protein exposure to animals from consumption of MON 88302 in feeds was evaluated by calculating an estimate of daily dietary intake (DDI). The highest percentage of CP4 EPSPS protein (g/kg bw) per total protein consumed was in the dairy cow, 0.0141% (g/g) of the total dietary protein intake (0.00084 g CP4 EPSPS/kg bw divided by 6 g dietary protein which is the total dietary protein intake for the cow). The chicken and pig percentages of the CP4 EPSPS protein consumed as part of the daily protein intake are much less than for the dairy cow. At the most, poultry, swine and lactating dairy cattle would be consuming less than 0.015% (g/g) of their total protein intake as CP4 EPSPS protein from MON 88302. Therefore, there is minimal exposure to MON 88302 CP4 EPSPS in relation to the total protein consumed.

V.F. CP4 EPSPS Protein Characterization and Safety Conclusion

The data and information provided in this section address the questions important to the food and feed safety of the CP4 EPSPS protein in MON 88302 including its potential allergenicity and toxicity. To summarize, the physicochemical characteristics of the CP4 EPSPS protein were determined and shown to be equivalent to those of an *E. coli*-produced CP4 EPSPS protein. The expression levels of the CP4 EPSPS protein in selected tissues of MON 88302 were determined. An assessment of the allergenic potential of the CP4 EPSPS protein supports the conclusion that the CP4 EPSPS protein does not pose a significant allergenic risk to humans or animals. In addition, donor organism for the CP4 EPSPS coding sequence, *Agrobacterium* sp. strain CP4, is ubiquitous in the environment and is not commonly known for human or animal

pathogenicity, or allergenicity. The CP4 EPSPS protein lacks structural similarity to allergens, toxins or other proteins known to have adverse effects on mammals. The CP4 EPSPS protein is rapidly digested in simulated digestive fluids and demonstrates no oral toxicity in mice at the level tested. Based on the above information, the consumption of the CP4 EPSPS protein from MON 88302 or its progeny are considered safe for humans and animals.

The protein safety data presented herein support the conclusion that food and feed products containing MON 88302 or derived from MON 88302 are as safe as canola currently on the market for human and animal consumption.

VI. COMPOSITIONAL ASSESSMENT OF MON 88302

Several Roundup Ready crops that produce the CP4 EPSPS protein have been reviewed by the USDA. The CP4 EPSPS protein expressed in MON 88302 is identical to the CP4 EPSPS protein in other Roundup Ready crops and the mode of action of CP4 EPSPS protein is well understood. Previous Roundup Ready crops reviewed by the USDA have had no biologically relevant compositional changes identified, and there is no reason to expect the CP4 EPSPS protein in MON 88302 would affect nutritionally important nutrients, toxicants, or anti-nutrients present in seed from this new product.

Safety assessments of biotechnology-derived crops follow the comparative safety assessment process (Codex Alimentarius, 2009) in which the composition of grain and/or other raw agricultural commodities of the biotechnology-derived crop are compared to the appropriate conventional counterpart that has a history of safe use. Compositional assessments are performed using the principles and analytes outlined in the OECD consensus document for canola composition (OECD, 2001).

A recent review of compositional assessments conducted according to OECD guidelines that encompassed a total of seven biotechnology-derived crop varieties, nine countries and eleven growing seasons concluded that incorporation of biotechnology-derived agronomic traits has had little impact on natural variation in crop composition. Most compositional variation is attributable to growing region, agronomic practices, and genetic background (Harrigan et al., 2010). Compositional quality, therefore, implies a very broad range of endogenous levels of individual constituents. Numerous scientific publications have further documented the extensive variability in the concentrations of crop nutrients, anti-nutrients, and secondary metabolites that reflect the influence of environmental and genetic factors as well as extensive conventional breeding efforts to improve nutrition, agronomics, and yield (Harrigan et al., 2010; Mailer and Pratley, 1990; Marwede et al., 2004; Naczk et al., 1998; OECD, 2001; Pritchard et al., 2000; Reynolds et al., 2005; Ridley et al., 2004; Werteker et al., 2010).

Compositional equivalence between biotechnology-derived and conventional crops provides an "equal or increased assurance of the safety of foods derived from genetically modified plants" (OECD, 1998). OECD consensus documents on compositional considerations for new crop varieties emphasize quantitative measurements of essential nutrients and known anti-nutrients. This is based on the premise that such comprehensive and detailed analyses will most effectively discern any compositional changes that imply potential nutritional or safety (*e.g.*, anti-nutritional) concerns. Levels of the components in seed and/or other raw agricultural commodities of the biotechnology-derived crop are compared to: 1) corresponding levels in a conventional comparator, the genetically similar conventional line, grown concurrently, in the same field trial, and 2) natural ranges generated from an evaluation of commercial reference varieties grown concurrently and from data published in the scientific literature. The comparison to data published in the literature places any potential differences between the assessed crop and its comparator in the context of the well-documented variation in the concentrations of crop nutrients, toxicants, and anti-nutrients.

This section provides analyses of concentrations of key nutrients, toxicants, and anti-nutrients of MON 88302 compared with equivalent analyses of a conventional counterpart grown and harvested under the same conditions, as appropriate. In addition, commercial conventional canola reference varieties were included in the composition analyses to establish a range of natural variability for each analyte, defined by a 99% tolerance interval. The production of materials for the compositional analyses used field designs to allow accurate assessments of compositional characteristics over a range of environmental conditions under which MON 88302 is expected to be grown. Design parameters included a sufficient number of trial sites to allow adequate exposure to the variety of conditions met in nature. Field sites were replicated with an adequate number of plant samples, and the methods of analysis were sufficiently sensitive and specific to detect variations in the components measured and to allow statistically rigorous analyses. The information provided in this section also addresses the relevant factors in Codex Plant Guidelines, Section 4, paragraphs 44 and 45 for compositional analyses (Codex Alimentarius, 2009).

VI.A. Compositional Equivalence of MON 88302 Seed to Conventional Canola

Compositional analysis comparing MON 88302 to the conventional control variety (Ebony) and commercial reference varieties demonstrated that MON 88302 is compositionally equivalent to conventional canola. Seed samples were collected from MON 88302 and the conventional control grown in a 2009 North American field production. Canola forage is rarely consumed by animals and is not a source of nutrition for humans. Therefore, the OECD consensus document on compositional considerations for canola (OECD, 2001) does not recommend analysis of canola forage, and forage samples were not analyzed. The background genetics of the conventional control were similar to that of MON 88302, but did not contain the *cp4 epsps* expression cassette. Seven different commercial reference varieties were included across all sites of the field production to provide data on natural variability of each compositional component analyzed. The samples utilized for compositional analysis were obtained from two U.S. sites [Wilkin County, Minnesota (MNCA) and McHenry County, North Dakota (NDVA)] and three Canadian sites [Portage la Prairie, Manitoba (MBPL); Newton, Manitoba (MBNW); and Saskatoon, Saskatchewan (SKSA)]. The sites were planted in a randomized complete block design with four replicates per site. MON 88302, the conventional control, and commercial reference varieties were treated with maintenance pesticides as necessary throughout the growing season. In addition to the conventional weed control programs, MON 88302 plots were treated at the 5-6 leaf stage with a glyphosate application at a target rate of 1.6 lb acid equivalents per acre (1800 g a.e./ha).

Compositional analyses were conducted as recommended for canola seed (OECD, 2001) to assess whether levels of key nutrients, toxicants and anti-nutrients in MON 88302 were equivalent to levels in the conventional control and to the composition of commercial reference varieties. Nutrients assessed in this analysis included proximates (ash, carbohydrates by calculation, moisture, protein, and total fat), fibers (acid detergent fiber [ADF], neutral detergent fiber [NDF], total dietary fiber [TDF]), amino acids (18 components), fatty acids (FA; C8-C24,), vitamin E (α-tocopherol), and minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and

zinc) in seed. The toxicants assessed in seed included erucic acid and glucosinolates (alkyl glucosinolates [including 3-butenyl, 4-pentenyl, 2-hydroxy-3-butenyl, and 2-hydroxy-4-pentenyl glucosinolates], indolyl glucosinolates [including 3-indolylmethyl and 4-hydroxy-3-indoylmethyl], and total glucosinolates [sum of alkyl and indolyl]). The anti-nutrients assessed in seed included phytic acid and sinapine (as sinapic acid). Methods used in the assessments of nutrients, toxicants and anti-nutrients are found in Appendix E. The toxicant and anti-nutrient results are discussed together under the general heading of anti-nutrients. In all, 70 different components were measured. Of those 70 components, 18 nutrients and one toxicant (18 fatty acids, including erucic acid, and one mineral) had more than 50% of the observations below the assay limit of quantitation (LOQ) and, as a result, were excluded from the statistical analyses. Therefore, 51 components were statistically assessed using a mixed model analysis of variance method. Values for all components were expressed on a dry weight basis with the exception of moisture, expressed as percent fresh weight, and fatty acids, expressed as percent of total FA.

For MON 88302, six statistical comparisons to the conventional control were conducted for each compositional component. One comparison was based on compositional data combined across all five field sites (combined-site analysis) and five separate comparisons were conducted on data from each of the individual field sites. Statistically significant differences were identified at the 5% level ($\alpha = 0.05$). Data from the commercial reference varieties were combined across all sites and used to calculate a 99% tolerance interval for each compositional component to define the natural variability of each component in canola varieties that have a history of safe consumption, and that were grown concurrently with MON 88302 and the conventional control in the same trial.

For the combined-site analysis, significant differences in nutrient, toxicant, and anti-nutrient components were further evaluated using considerations relevant to the safety and nutritional quality of MON 88302 when compared to the conventional control, which is the conventional counterpart that has a history of safe consumption. Considerations used to assess the relevance of each combined-site statistically significant difference included: 1) the relative magnitude of the difference in the mean values of nutrient, toxicant, and anti-nutrient components of MON 88302 and the conventional control; 2) whether the MON 88302 component mean value is within the range of natural variability of that component as represented by the 99% tolerance interval of the commercial reference varieties grown concurrently in the same trial; 3) evaluation of the reproducibility of the statistically significant ($\alpha = 0.05$) combined-site component differences at individual sites; and 4) an assessment of the differences within the context of natural variability of commercial canola composition published in the scientific literature. If statistically significant differences detected in the individual site analyses were not observed in the combined-site analysis, they were not considered further for the compositional assessment of safety. Statistical summaries of nutrients, toxicants and anti-nutrients for individual sites are found in Appendix E.

This analysis provides a comprehensive comparative assessment of the levels of key nutrients, toxicants, and anti-nutrients in seed of MON 88302 and the conventional control, discussed in the context of natural variability in composition of commercial

canola. Results of the comparison indicate that the composition of the seed of MON 88302 is equivalent to that of the conventional control and within the natural variability of commercial reference varieties.

VI.A.1. Nutrient Levels in Canola Seed

In the combined-site analysis of nutrient levels in seed, the following components showed no significant differences in mean values between MON 88302 and the conventional control: proximates, two types of fiber (ADF and NDF), 18 amino acids (alanine, arginine, aspartic acid, cystine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine), four fatty acids (16:0 palmitic acid, 20:1 eicosenoic acid, 24:0 lignoceric acid, and 24:1 nervonic acid), eight minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, and zinc), and vitamin E (Table VI-2).

The components that showed significant differences in mean values between MON 88302 and the conventional control in the combined-site analysis were: total dietary fiber (TDF) and seven fatty acids (16:1 palmitoleic acid, 18:0 stearic acid, 18:1 oleic acid, 18:2 linoleic acid, 18:3 linolenic acid, 20:0 arachidic acid, and 22:0 behenic acid) (Tables VI-1 and VI-2).

1) The statistically significant differences in nutrients were evaluated using considerations relevant to the nutritional quality of MON 88302 when compared to the conventional control: eight combined-site nutrient significant differences ($\alpha = 0.05$) between MON 88302 and the conventional control were attributable to TDF (expressed as % dry weight) and seven fatty acids (expressed as % total FA). The relative magnitudes of differences between the combined-site mean values for MON 88302 and the conventional control showed an increase for TDF, 18:2 linoleic acid, and 18:3 linolenic acid, (13.81%, 8.98%, and 20.01%, respectively) and a decrease for 16:1 palmitoleic acid, 18:0 stearic acid, 18:1 oleic acid, 20:0 arachidic acid, and 22:0 behenic acid (7.56%, 15.06%, 4.52%, 10.68%, and 6.01%, respectively). The relative differences in these components in the combined-site analysis and at individual sites were between 3.48% and 28.69% (Table VI-2 and Tables E-3, E-5, E-7, E-9, and E-11). The magnitudes of differences observed between MON 88302 and the conventional control were small relative to the natural variability of these components as determined by the 99% tolerance interval established by the concurrently grown commercial reference varieties that have a history of safe consumption, as presented in the tables referenced above.

2) Mean values for all of the nutrient components found to be significantly different ($\alpha = 0.05$) from the combined-site analysis of MON 88302 were within the 99% tolerance interval established from the commercial reference varieties grown concurrently and were, therefore, within the range of natural variability of that component in commercial canola varieties that have a history of safe consumption (Table VI-1).

3) Assessment of the reproducibility of the combined-site differences at the five individual sites demonstrated no significant differences for TDF; however, significant differences ($\alpha = 0.05$) were observed for 18:0 stearic acid, 18:1 oleic acid, and 18:2 linoleic acid at all five sites; significant differences for 16:1 palmitoleic acid and 18:3 linolenic acid at four sites, significant differences for 20:0 arachidic acid at three sites, and significant differences for 22:0 behenic acid at two sites (Table VI-1). The magnitudes of differences between the mean fatty acid values for MON 88032 and the conventional control were small relative to the variability of these components as determined by the 99% tolerance interval established by the concurrently grown commercial reference varieties, and relative to the variability of fatty acid components in canola due to environment (Pritchard et al., 2000). Individual site mean values of MON 88302 for all nutrient components with significant differences fell within the 99% tolerance interval established from the commercial reference varieties grown concurrently and were, therefore, within the range of natural variability of that component in commercial canola varieties that have a history of safe consumption (Table VI-2).

4) With the exception of TDF, for which no commercial reference values have been published, all of the compositional components identified as significantly different from the conventional control were within the natural variability of these components in commercial canola composition as published in the scientific literature (Table VI-4).

In summary, the combined-site statistical analysis identified eight significant differences ($\alpha = 0.05$) that were small in magnitude relative to their natural variability as determined by the 99% tolerance interval established by the concurrently grown commercial reference varieties that have a history of safe consumption. Of these significant differences, only 18:0 stearic acid, 18:1 oleic acid, and 18:2 linoleic acid were observed consistently at all of the individual sites. All of the components identified as significantly different in the combined-site analysis and corresponding individual site analyses, were within the natural variability of commercial canola defined by the 99% tolerance interval established by the concurrently grown commercial reference varieties, and were within the published literature ranges (there is no published reference data for TDF) for these components in conventional canola. Therefore, these significant differences are not meaningful to food and feed safety or nutrition. These findings support the conclusion that nutrients in seed from MON 88302 are compositionally equivalent to those in conventional canola varieties with a history of safe usage.

VI.A.2. Anti-Nutrient Levels in Canola Seed

According to OECD (2001), canola seed contains toxicants including erucic acid and glucosinolates, and anti-nutrients, including phytic acid and sinapine. Erucic acid has been shown to have cardiopathic potential resulting in a weakening of the heart muscle in experimental animals (Bozcali et al., 2009; Chien et al., 1983). Glucosinolates in canola seed can be characterized into two main chemical groups, alkyl and indolyl, with alkyl being the most common (CCC, 2009). Upon enzymatic hydrolysis with myrosinase, certain glucosinolates form compounds that can depress growth and thyroid function

(Bell, 1984). The standard for glucosinolates in canola seed is <18 µmoles/g (Szmigielska et al., 2000). Phytic acid is present in canola seed. Phytic acid chelates mineral nutrients, including calcium, magnesium, potassium, iron, and zinc, rendering them biologically unavailable to monogastric animals consuming the seed (Liener, 2000). Sinapine is the choline ester of sinapic acid, the primary phenolic component in canola seed. Sinapine imparts a bitter taste and reduces palatability of the seed (OECD, 2001). Sinapine levels were determined based on quantitation of the hydrolysis product, sinapic acid.

MON 88302 levels of 22:1 erucic acid were below the level of detection (0.04% total FA) in canola seed, and therefore, 22:0 erucic acid was excluded from statistical analysis. In the combined-site analysis, no significant difference ($\alpha = 0.05$) was observed between MON 88302 and the conventional control (Tables VI-1 and VI-3) for indolyl glucosinolates, total glucosinolates, phytic acid, and sinapine. One statistically significant difference was identified for alkyl glucosinolates, and the net effect was a slight reduction of this anti-nutrient in MON 88302. The following considerations show that this difference is not a meaningful concern from a food and feed nutritional or safety perspective:

1) The magnitude of the difference between the combined-site mean value for alkyl glucosinolates in MON 88302 and the conventional control showed a 27.59% decrease. This magnitude of difference was small relative to the natural variability of these components as determined by the 99% tolerance interval established by the concurrently grown commercial reference varieties that have a history of safe consumption.

2) The MON 88302 mean alkyl glucosinolates value from the combined-site analysis was within the 99% tolerance interval established from the commercial reference varieties grown concurrently. The mean value was, therefore, within the range of natural variability for alkyl glucosinolates in commercial canola varieties that have a history of safe consumption (Tables VI-1 and VI-3).

3) The combined-site difference for alkyl glucosinolates was not consistently observed at all individual sites. A significant difference for alkyl glucosinolates was observed at one of the individual sites. However, the mean value for alkyl glucosinolates in MON 88302 at this individual site was within the 99% tolerance interval established from the concurrently grown commercial reference varieties.

4) An assessment based on of the natural variability of alkyl glucosinolates in commercial canola varieties could not be made because a range was not available in the scientific literature.

In summary, the statistical analyses found a combined-site significant difference in alkyl glucosinolates that was lower than the conventional mean value, and not consistently observed at the individual sites. The mean alkyl glucosinolates value for MON 88302 was within the natural variability of commercial canola defined by the 99% tolerance interval established from the concurrently grown commercial reference varieties that have

a history of safe consumption, and the value was within the safety threshold for canola. Total glucosinolate levels in seed from MON 88302 ranged from 1.73 to 11.42 µmoles/g (Table VI-3 and Tables E-4, E-6, E-8, E-10, and E-12), within the standard for canola. Thus, an evaluation of anti-nutrient components in seed supports the conclusion that MON 88302 is as safe as and compositionally equivalent to conventional canola.

Table VI-1. Summary of Differences (p < 0.05) for the Comparison of Canola Seed Component Levels for MON 88302 vs. the Conventional Control

Analytical Component (Units)[1]	MON 88302[2] Mean[3]	Control[4] Mean	Mean Difference (MON 88302 minus Control) Mean Difference (% of Control)	Mean Difference (MON 88302 minus Control) Significance (p-Value)	MON 88302 Range	Commercial Tolerance Interval[5]
Statistical Differences Observed in Combined-Site Analysis						
Seed Fiber (% dw)						
Total Dietary Fiber	20.90	18.37	13.81	0.004	16.91 - 27.81	13.97, 24.85
Seed Fatty Acid (% Total FA)						
16:1 Palmitoleic	0.22	0.24	-7.56	0.008	0.20 - 0.26	0.17, 0.30
18:0 Stearic	1.68	1.98	-15.06	<0.001	1.54 - 1.87	0.90, 3.05
18:1 Oleic	62.82	65.79	-4.52	<0.001	60.51 - 65.20	56.13, 70.69
18:2 Linoleic	19.26	17.67	8.98	<0.001	17.78 - 20.66	12.60, 24.49
18:3 Linolenic	9.58	7.98	20.01	<0.001	8.71 - 11.23	6.96, 11.73
20:0 Arachidic	0.54	0.60	-10.68	<0.001	0.50 - 0.57	0.45, 0.80
22:0 Behenic	0.27	0.28	-6.01	0.016	0.24 - 0.29	0.19, 0.43

Table VI-1. Summary of Differences (p<0.05) for the Comparison of Canola Seed Component Levels for MON 88302 vs. the Conventional Control (continued)

Analytical Component (Units)[1]	MON 88302[2] Mean[3]	Control[4] Mean	Mean Difference (MON 88302 minus Control) Mean Difference (% of Control)	Mean Difference (MON 88302 minus Control) Significance (p-Value)	MON 88302 Range	Commercial Tolerance Interval[5]
Statistical Differences Observed in Combined-Site Analysis						
Seed Anti-nutrient						
Alkyl Glucosinolate (µmole/g dw)	3.68	5.08	-27.59	0.035	1.19 - 5.87	0, 29.02
Statistical Differences Observed in More than One Individual Site						
Seed Fatty Acid (% Total FA)						
18:0 Stearic Site MBNW	1.73	1.97	-12.23	0.028	1.64 - 1.87	0.90, 3.05
18:0 Stearic Site MBPL	1.58	1.87	-15.64	<0.001	1.55 - 1.59	0.90, 3.05
18:0 Stearic Site MNCA	1.67	1.86	-10.01	0.022	1.65 - 1.71	0.90, 3.05
18:0 Stearic Site NDVA	1.77	2.11	-16.06	0.004	1.71 - 1.84	0.90, 3.05
18:0 Stearic Site SKSA	1.66	2.08	-20.14	0.001	1.54 - 1.72	0.90, 3.05
18:1 Oleic Site MBNW	63.40	65.71	-3.51	0.004	62.94 - 64.03	56.13, 70.69

Table VI-1. Summary of Differences (p<0.05) for the Comparison of Canola Seed Component Levels for MON 88302 vs. the Conventional Control (continued)

Analytical Component (Units)[1]	MON 88302[2] Mean[3]	Control[4] Mean	Mean Difference (MON 88302 minus Control) Mean Difference (% of Control)	Mean Difference (MON 88302 minus Control) Significance (p-Value)	MON 88302 Range	Commercial Tolerance Interval[5]
Statistical Differences Observed in More than One Individual Site						
Seed Fatty Acid (% Total FA)						
18:1 Oleic Site MBPL	62.06	64.30	-3.48	<0.001	61.82 - 62.35	56.13, 70.69
18:1 Oleic Site MNCA	61.67	64.86	-4.92	0.005	61.70 - 61.87	56.13, 70.69
18:1 Oleic Site NDVA	65.14	68.38	-4.74	0.003	64.90 - 65.20	56.13, 70.69
18:1 Oleic Site SKSA	61.91	65.69	-5.75	0.001	60.51 - 62.29	56.13, 70.69
18:2 Linoleic Site MBNW	19.27	17.89	7.71	0.011	18.82 - 19.66	12.60, 24.49
18:2 Linoleic Site MBPL	20.43	19.18	6.50	<0.001	20.13 - 20.66	12.60, 24.49
18:2 Linoleic Site MNCA	20.20	18.35	10.07	0.001	20.00 - 20.32	12.60, 24.49
18:2 Linoleic Site NDVA	17.86	15.71	13.67	0.009	17.78 - 18.02	12.60, 24.49

Table VI-1. Summary of Differences (p<0.05) for the Comparison of Canola Seed Component Levels for MON 88302 vs. the Conventional Control (continued)

Analytical Component (Units)[1]	MON 88302[2] Mean[3]	Control[4] Mean	Mean Difference (MON 88302 minus Control) Mean Difference (% of Control)	Mean Difference (MON 88302 minus Control) Significance (p-Value)	MON 88302 Range	Commercial Tolerance Interval[5]
Statistical Differences Observed in More than One Individual Site						
Seed Fatty Acid (% Total FA)						
18:2 Linoleic Site SKSA	18.49	17.22	7.36	0.019	18.08 - 19.48	12.60, 24.49
Seed Vitamin (mg/100g dw)						
Vitamin E (a-tocopherol) Site MBNW	13.06	9.36	39.51	0.004	12.22 - 13.47	3.88, 17.28
Vitamin E (α-tocopherol) Site MBPL	11.50	7.63	50.83	<0.001	10.70 - 12.20	3.88, 17.28
Vitamin E (α-tocopherol) Site MNCA	13.39	10.82	23.73	0.006	12.58 - 14.62	3.88, 17.28
Vitamin E (α-tocopherol) Site NDVA	15.89	9.43	68.39	0.010	15.23 - 16.55	3.88, 17.28
Vitamin E (α-tocopherol) Site SKSA	1.49	6.91	-78.47	0.019	1.30 - 1.66	3.88, 17.28
Seed Anti-nutrient						
Sinapic Acid (% dw) Site MBNW	1.02	0.92	10.34	0.001	0.99 - 1.06	0.57, 1.13

Table VI-1. Summary of Differences (p<0.05) for the Comparison of Canola Seed Component Levels for MON 88302 vs. the Conventional Control (continued)

Analytical Component (Units)[1]	MON 88302[2] Mean[3]	Control[4] Mean	Mean Difference (MON 88302 minus Control) Mean Difference (% of Control)	Mean Difference (MON 88302 minus Control) Significance (p-Value)	MON 88302 Range	Commercial Tolerance Interval[5]
Statistical Differences Observed in More than One Individual Site						
Seed Anti-nutrient						
Sinapic Acid (% dw) Site MBPL	0.97	0.86	12.04	<0.001	0.95 - 0.99	0.57, 1.13
Sinapic Acid (% dw) Site MNCA	1.06	0.96	10.66	0.001	1.02 - 1.08	0.57, 1.13
Sinapic Acid (% dw) Site NDVA	1.02	0.83	23.56	0.001	1.00 - 1.04	0.57, 1.13
Sinapic Acid (% dw) Site SKSA	0.22	0.81	-73.12	0.001	0.16 - 0.28	0.57, 1.13
Seed Fatty Acid (% Total FA)						
16:1 Palmitoleic Site MBNW	0.21	0.23	-9.71	0.015	0.20 - 0.21	0.17, 0.30
16:1 Palmitoleic Site MBPL	0.23	0.25	-10.10	0.008	0.22 - 0.23	0.17, 0.30
16:1 Palmitoleic Site MNCA	0.21	0.24	-10.88	0.001	0.21 - 0.21	0.17, 0.30
16:1 Palmitoleic Site NDVA	0.20	0.22	-11.05	0.036	0.20 - 0.20	0.17, 0.30

Table VI-1. Summary of Differences (p<0.05) for the Comparison of Canola Seed Component Levels for MON 88302 vs. the Conventional Control (continued)

Analytical Component (Units)[1]	MON 88302[2] Mean[3]	Control[4] Mean	Mean Difference (MON 88302 minus Control) Mean Difference (% of Control)	Mean Difference (MON 88302 minus Control) Significance (p-Value)	MON 88302 Range	Commercial Tolerance Interval[5]
Statistical Differences Observed in More than One Individual Site						
Seed Fatty Acid (% Total FA)						
18:3 Linolenic Site MBNW	9.19	8.12	13.27	0.004	8.88 - 9.42	6.96, 11.73
18:3 Linolenic Site MBPL	9.28	7.74	19.89	<0.001	9.12 - 9.43	6.96, 11.73
18:3 Linolenic Site NDVA	8.82	7.31	20.67	<0.001	8.71 - 8.94	6.96, 11.73
18:3 Linolenic Site SKSA	10.78	8.38	28.69	<0.001	10.39 - 11.23	6.96, 11.73
Seed Fatty Acid (% Total FA)						
20:0 Arachidic Site MBPL	0.53	0.60	-11.73	<0.001	0.52 - 0.54	0.45, 0.80
20:0 Arachidic Site NDVA	0.57	0.65	-12.58	<0.001	0.56 - 0.57	0.45, 0.80
20:0 Arachidic Site SKSA	0.54	0.62	-13.28	<0.001	0.52 - 0.55	0.45, 0.80

Table VI-1. Summary of Differences (p<0.05) for the Comparison of Canola Seed Component Levels for MON 88302 vs. the Conventional Control (continued)

Analytical Component (Units)[1]	MON 88302[2] Mean[3]	Control[4] Mean	Mean Difference (MON 88302 minus Control) Mean Difference (% of Control)	Mean Difference (MON 88302 minus Control) Significance (p-Value)	MON 88302 Range	Commercial Tolerance Interval[5]
Statistical Differences Observed in More than One Individual Site						
Seed Mineral						
Copper (mg/kg dw) Site MBNW	3.72	3.41	9.28	0.013	3.61 - 3.83	2.00, 4.43
Copper (mg/kg dw) Site MBPL	3.47	3.97	-12.50	0.016	3.35 - 3.56	2.00, 4.43
Copper (mg/kg dw) Site MNCA	4.40	4.11	6.91	0.027	4.16 - 4.57	2.00, 4.43
Seed Fatty Acid (% Total FA)						
22:0 Behenic Site MBPL	0.27	0.30	-13.00	<0.001	0.26 - 0.27	0.19, 0.43
22:0 Behenic Site NDVA	0.27	0.30	-9.83	0.007	0.27 - 0.27	0.19, 0.43
Seed Mineral						
Iron (mg/kg dw) Site MBPL	44.13	51.01	-13.48	0.001	42.80 - 45.09	23.39, 86.23
Iron (mg/kg dw) Site MNCA	42.57	50.64	-15.93	0.007	40.56 - 44.18	23.39, 86.23

Table VI-1. Summary of Differences (p<0.05) for the Comparison of Canola Seed Component Levels for MON 88302 vs. the Conventional Control (continued)

Analytical Component (Units)[1]	MON 88302[2] Mean[3]	Control[4] Mean	Mean Difference (MON 88302 minus Control) Mean Difference (% of Control)	Mean Difference (MON 88302 minus Control) Significance (p-Value)	MON 88302 Range	Commercial Tolerance Interval[5]
Statistical Differences Observed in More than One Individual Site						
Seed Mineral						
Potassium (g/100g dw) Site MBPL	0.70	0.77	-8.91	0.023	0.63 - 0.76	0.39, 0.96
Potassium (g/100g dw) Site SKSA	0.82	0.71	15.32	<0.001	0.77 - 0.90	0.39, 0.96
Zinc (mg/kg dw) Site MBPL	31.25	33.88	-7.76	0.024	30.45 - 32.05	20.19, 48.23
Zinc (mg/kg dw) Site SKSA	41.58	33.10	25.61	0.010	39.33 - 45.49	20.19, 48.23
Statistical Differences Observed in One Individual Site						
Seed Proximate (% dw)						
Carbohydrates Site MNCA	27.31	25.99	5.07	0.035	26.27 - 27.90	23.12, 30.77
Moisture (% fw) Site MNCA	5.52	6.69	-17.46	<0.001	5.37 - 5.61	4.33, 6.91
Protein Site SKSA	23.82	22.14	7.58	0.038	23.62 - 24.58	17.20, 30.08

Table VI-1. Summary of Differences (p<0.05) for the Comparison of Canola Seed Component Levels for MON 88302 vs. the Conventional Control (continued)

Analytical Component (Units)[1]	MON 88302[2] Mean[3]	Control[4] Mean	Mean Difference (MON 88302 minus Control) Mean Difference (% of Control)	Mean Difference (MON 88302 minus Control) Significance (p-Value)	MON 88302 Range	Commercial Tolerance Interval[5]
Statistical Differences Observed in One Individual Site						
Seed Proximate (% dw)						
Total Fat Site NDVA	48.04	45.17	6.35	0.014	47.20 - 48.87	39.65, 51.24
Seed Fiber (% dw)						
Acid Detergent Fiber Site MBPL	16.75	14.19	18.00	0.005	15.17 - 18.19	6.95, 23.92
Neutral Detergent Fiber Site MBPL	19.45	16.87	15.31	0.017	18.35 - 20.02	10.07, 25.94
Seed Amino Acid (% dw)						
Tyrosine Site MBPL	0.72	0.71	2.46	0.028	0.72 - 0.73	0.57, 0.81
Valine Site MNCA	1.15	1.24	-7.32	0.048	1.13 - 1.15	0.92, 1.55
Seed Fatty Acid (% Total FA)						
16:0 Palmitic Site SKSA	4.51	4.07	10.90	<0.001	4.46 - 4.57	2.84, 5.26
20:1 Eicosenoic Site SKSA	1.24	1.13	9.55	0.005	1.22 - 1.26	0.83, 1.68

Table VI-1. Summary of Differences (p<0.05) for the Comparison of Canola Seed Component Levels for MON 88302 vs. the Conventional Control (continued)

Analytical Component (Units)[1]	MON 88302[2] Mean[3]	Control[4] Mean	Mean Difference (MON 88302 minus Control) Mean Difference (% of Control)	Mean Difference (MON 88302 minus Control) Significance (p-Value)	MON 88302 Range	Commercial Tolerance Interval[5]
Statistical Differences Observed in One Individual Site						
Seed Fatty Acid (% Total FA)						
24:0 Lignoceric Site MBPL	0.16	0.19	-12.24	0.029	0.16 - 0.17	0.033, 0.25
24:1 Nervonic Site MBPL	0.13	0.16	-20.37	0.031	0.12 - 0.13	0.041, 0.18
Seed Anti-nutrient						
Alkyl Glucosinolate (µmole/g dw) Site SKSA	1.61	5.82	-72.32	0.005	1.19 - 2.17	0, 29.02
Indolyl Glucosinolate (µmole/g dw) Site SKSA	0.86	3.30	-73.88	0.001	0.49 - 1.31	1.37, 6.62
Total Glucosinolate (µmole/g dw) Site SKSA	2.53	9.22	-72.58	0.002	1.73 - 3.51	0, 32.20

[1]dw = dry weight; fw = fresh weight; FA = fatty acid.
[2]MON 88302 treated with glyphosate.
[3]Mean = least-square mean.
[4]Control refers to the genetically similar, conventional control Ebony.
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial conventional reference varieties. Negative limits set to zero.

Table VI-2. Statistical Summary of Combined-site Canola Seed Nutrients for MON 88302 vs. Conventional Control

Analytical Component (Units)[1]	MON 88302[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88302 minus Control) Mean (S.E.) (Range)	95% CI[3] Lower, Upper	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Proximate (% dw)						
Ash	3.96 (0.18) (3.31 - 4.45)	3.90 (0.18) (3.20 - 5.10)	0.055 (0.095) (-0.21 - 0.64)	-0.14, 0.25	0.565	3.32, 4.66 (2.98 - 4.52)
Carbohydrates	25.96 (0.68) (21.83 - 28.81)	26.13 (0.68) (23.91 - 28.73)	-0.17 (0.54) (-4.18 - 1.94)	-1.42, 1.09	0.765	23.12, 30.77 (22.53 - 29.96)
Moisture (% fw)	5.35 (0.34) (3.90 - 6.08)	5.45 (0.34) (4.41 - 6.98)	-0.10 (0.24) (-1.53 - 0.87)	-0.65, 0.45	0.688	4.33, 6.91 (4.09 - 8.48)
Protein	23.04 (0.70) (19.68 - 25.98)	23.14 (0.69) (20.29 - 27.02)	-0.10 (0.52) (-2.29 - 2.50)	-1.32, 1.11	0.847	17.20, 30.08 (18.68 - 28.32)
Total Fat	47.06 (0.83) (43.96 - 49.26)	46.82 (0.83) (43.65 - 50.24)	0.24 (0.52) (-2.28 - 4.10)	-1.00, 1.48	0.659	39.65, 51.24 (40.71 - 50.26)
Fiber (% dw)						
Acid Detergent Fiber	15.32 (1.36) (9.19 - 20.24)	14.47 (1.36) (8.94 - 18.71)	0.84 (0.41) (-2.71 - 3.57)	-0.14, 1.83	0.082	6.95, 23.92 (9.75 - 21.22)

Table VI-2. Statistical Summary of Combined-Site Seed Nutrient Content for MON 88302 vs. the Conventional Control (continued)

Analytical Component (Units)[1]	MON 88302[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88302 minus Control) Mean (S.E.) (Range)	95% CI[3] Lower, Upper	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fiber (% dw)						
Neutral Detergent Fiber	17.43 (1.38) (9.48 - 21.36)	16.70 (1.38) (11.56 - 19.58)	0.74 (0.57) (-2.74 - 4.43)	-0.58, 2.05	0.231	10.07, 25.94 (10.93 - 22.75)
Total Dietary Fiber	20.90 (0.79) (16.91 - 27.81)	18.37 (0.78) (14.58 - 23.00)	2.54 (0.84) (-0.49 - 9.96)	0.85, 4.23	0.004	13.97, 24.85 (12.64 - 26.47)
Amino Acid (% dw)						
Alanine	1.02 (0.025) (0.88 - 1.15)	1.04 (0.025) (0.93 - 1.19)	-0.015 (0.022) (-0.12 - 0.069)	-0.066, 0.035	0.502	0.77, 1.34 (0.87 - 1.27)
Arginine	1.45 (0.054) (1.23 - 1.72)	1.51 (0.054) (1.29 - 1.77)	-0.063 (0.032) (-0.27 - 0.15)	-0.13, 0.00082	0.052	1.10, 1.93 (1.23 - 1.96)
Aspartic Acid	1.65 (0.067) (1.40 - 1.93)	1.71 (0.067) (1.46 - 1.97)	-0.055 (0.043) (-0.37 - 0.12)	-0.16, 0.045	0.238	1.33, 2.12 (1.42 - 2.23)
Cystine	0.57 (0.027) (0.48 - 0.73)	0.58 (0.027) (0.49 - 0.79)	-0.0044 (0.015) (-0.054 - 0.053)	-0.040, 0.031	0.781	0.38, 0.83 (0.45 - 0.79)

Table VI-2. Statistical Summary of Combined-Site Seed Nutrient Content for MON 88302 vs. the Conventional Control (continued)

Analytical Component (Units)[1]	MON 88302[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88302 minus Control) Mean (S.E.) (Range)	95% CI[3] Lower, Upper	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dw)						
Glutamic Acid	4.06 (0.18) (3.37 - 5.06)	4.24 (0.17) (3.64 - 5.26)	-0.19 (0.10) (-0.68 - 0.36)	-0.43, 0.049	0.103	2.73, 5.89 (3.26 - 5.43)
Glycine	1.14 (0.040) (1.02 - 1.32)	1.19 (0.040) (1.01 - 1.38)	-0.041 (0.025) (-0.18 - 0.044)	-0.10, 0.018	0.142	0.96, 1.47 (1.01 - 1.50)
Histidine	0.63 (0.023) (0.55 - 0.77)	0.65 (0.023) (0.57 - 0.78)	-0.015 (0.011) (-0.065 - 0.044)	-0.038, 0.0074	0.181	0.47, 0.86 (0.54 - 0.80)
Isoleucine	0.93 (0.028) (0.81 - 1.08)	0.96 (0.028) (0.82 - 1.12)	-0.024 (0.021) (-0.13 - 0.041)	-0.074, 0.026	0.299	0.70, 1.22 (0.78 - 1.15)
Leucine	1.64 (0.049) (1.40 - 1.90)	1.68 (0.049) (1.46 - 1.95)	-0.042 (0.039) (-0.25 - 0.086)	-0.13, 0.048	0.308	1.21, 2.18 (1.36 - 2.07)
Lysine	1.39 (0.041) (1.22 - 1.63)	1.41 (0.041) (1.25 - 1.65)	-0.019 (0.023) (-0.12 - 0.086)	-0.064, 0.027	0.410	1.02, 1.90 (1.20 - 1.68)

Table VI-2. Statistical Summary of Combined-Site Seed Nutrient Content for MON 88302 vs. the Conventional Control (continued)

Analytical Component (Units)[1]	MON 88302[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88302 minus Control) Mean (S.E.) (Range)	95% CI[3] Lower, Upper	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dw)						
Methionine	0.46 (0.015) (0.40 - 0.54)	0.46 (0.015) (0.40 - 0.56)	-0.0018 (0.0089) (-0.038 - 0.034)	-0.022, 0.019	0.847	0.30, 0.65 (0.36 - 0.57)
Phenylalanine	0.98 (0.029) (0.84 - 1.11)	1.00 (0.028) (0.87 - 1.15)	-0.024 (0.024) (-0.17 - 0.044)	-0.079, 0.031	0.348	0.77, 1.26 (0.84 - 1.25)
Proline	1.40 (0.054) (1.20 - 1.71)	1.42 (0.054) (1.20 - 1.73)	-0.028 (0.027) (-0.16 - 0.17)	-0.093, 0.036	0.335	0.90, 2.01 (1.12 - 1.78)
Serine	1.02 (0.030) (0.87 - 1.14)	1.05 (0.030) (0.94 - 1.18)	-0.035 (0.019) (-0.17 - 0.052)	-0.080, 0.0095	0.105	0.81, 1.32 (0.88 - 1.30)
Threonine	0.98 (0.030) (0.86 - 1.11)	1.00 (0.030) (0.88 - 1.12)	-0.025 (0.018) (-0.12 - 0.065)	-0.066, 0.016	0.192	0.82, 1.20 (0.84 - 1.22)
Tryptophan	0.23 (0.010) (0.17 - 0.26)	0.24 (0.010) (0.19 - 0.31)	-0.013 (0.0093) (-0.063 - 0.036)	-0.032, 0.0059	0.172	0.13, 0.35 (0.17 - 0.32)

Table VI-2. Statistical Summary of Combined-Site Seed Nutrient Content for MON 88302 vs. the Conventional Control (continued)

Analytical Component (Units)[1]	MON 88302[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88302 minus Control) Mean (S.E.) (Range)	95% CI[3] Lower, Upper l	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dw)						
Tyrosine	0.67 (0.019) (0.59 - 0.75)	0.69 (0.019) (0.61 - 0.77)	-0.017 (0.013) (-0.11 - 0.028)	-0.048, 0.015	0.249	0.57, 0.81 (0.60 - 0.84)
Valine	1.20 (0.035) (1.04 - 1.37)	1.22 (0.035) (1.05 - 1.41)	-0.025 (0.025) (-0.16 - 0.054)	-0.084, 0.034	0.352	0.92, 1.55 (1.01 - 1.46)
Fatty Acid (% Total FA)						
16:0 Palmitic	4.23 (0.078) (3.95 - 4.57)	4.10 (0.077) (3.94 - 4.41)	0.13 (0.067) (-0.22 - 0.48)	-0.027, 0.28	0.094	2.84, 5.26 (3.55 - 4.69)
16:1 Palmitoleic	0.22 (0.0081) (0.20 - 0.26)	0.24 (0.0081) (0.22 - 0.26)	-0.018 (0.0053) (-0.039 - 0.0074)	-0.030, -0.0059	0.008	0.17, 0.30 (0.19 - 0.27)
18:0 Stearic	1.68 (0.044) (1.54 - 1.87)	1.98 (0.044) (1.78 - 2.19)	-0.30 (0.031) (-0.48 - -0.059)	-0.37, -0.23	<0.001	0.90, 3.05 (1.50 - 2.64)
18:1 Oleic	62.82 (0.62) (60.51 - 65.20)	65.79 (0.62) (63.72 - 68.44)	-2.97 (0.31) (-4.30 - -1.52)	-3.69, -2.26	<0.001	56.13, 70.69 (57.86 - 68.53)

Table VI-2. Statistical Summary of Combined-Site Seed Nutrient Content for MON 88302 vs. the Conventional Control (continued)

Analytical Component (Units)[1]	MON 88302[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88302 minus Control) Mean (S.E.) (Range)	95% CI[3] Lower, Upper	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fatty Acid (% Total FA)						
18:2 Linoleic	19.26 (0.51) (17.78 - 20.66)	17.67 (0.51) (15.72 - 19.29)	1.59 (0.17) (0.40 - 2.42)	1.20, 1.97	<0.001	12.60, 24.49 (14.12 - 22.57)
18:3 Linolenic	9.58 (0.27) (8.71 - 11.23)	7.98 (0.27) (7.19 - 8.99)	1.60 (0.21) (0.76 - 2.64)	1.12, 2.07	<0.001	6.96, 11.73 (7.99 - 10.94)
20:0 Arachidic	0.54 (0.011) (0.50 - 0.57)	0.60 (0.011) (0.54 - 0.65)	-0.064 (0.0074) (-0.091 - -0.0032)	-0.081, -0.047	<0.001	0.45, 0.80 (0.53 - 0.71)
20:1 Eicosenoic	1.13 (0.024) (1.06 - 1.26)	1.09 (0.024) (1.00 - 1.18)	0.036 (0.017) (-0.042 - 0.14)	-0.0034, 0.076	0.068	0.83, 1.68 (1.04 - 1.56)
22:0 Behenic	0.27 (0.0072) (0.24 - 0.29)	0.28 (0.0072) (0.24 - 0.31)	-0.017 (0.0056) (-0.047 - 0.016)	-0.030, -0.0041	0.016	0.19, 0.43 (0.27 - 0.38)
24:0 Lignoceric	0.16 (0.016) (0.049 - 0.23)	0.16 (0.015) (0.045 - 0.22)	0.0038 (0.017) (-0.14 - 0.11)	-0.030, 0.038	0.823	0.033, 0.25 (0.044 - 0.21)

Table VI-2. Statistical Summary of Combined-Site Seed Nutrient Content for MON 88302 vs. the Conventional Control (continued)

Analytical Component (Units)[1]	MON 88302[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88302 minus Control) Mean (S.E.) (Range)	95% CI[3] Lower, Upper	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fatty Acid (% Total FA)						
24:1 Nervonic	0.12 (0.015) (0.046 - 0.20)	0.11 (0.015) (0.045 - 0.17)	0.013 (0.014) (-0.072 - 0.081)	-0.020, 0.047	0.377	0.041, 0.18 (0.044 - 0.20)
Mineral						
Calcium (g/100g dw)	0.41 (0.030) (0.30 - 0.51)	0.40 (0.030) (0.28 - 0.49)	0.015 (0.012) (-0.068 - 0.081)	-0.0089, 0.039	0.210	0.16, 0.61 (0.25 - 0.53)
Copper (mg/kg dw)	3.78 (0.17) (3.27 - 4.57)	3.65 (0.17) (2.96 - 4.18)	0.14 (0.14) (-0.83 - 0.57)	-0.19, 0.46	0.361	2.00, 4.43 (2.52 - 4.93)
Iron (mg/kg dw)	48.73 (4.28) (40.55 - 69.61)	54.01 (4.24) (41.65 - 77.74)	-5.28 (2.89) (-20.41 - 14.87)	-11.85, 1.30	0.102	23.39, 86.23 (39.16 - 77.92)
Magnesium (g/100g dw)	0.37 (0.014) (0.31 - 0.42)	0.36 (0.014) (0.31 - 0.42)	0.0048 (0.0070) (-0.032 - 0.043)	-0.011, 0.021	0.508	0.32, 0.43 (0.30 - 0.45)
Manganese (mg/kg dw)	41.44 (2.02) (35.28 - 51.55)	40.34 (1.99) (33.12 - 50.97)	1.10 (1.83) (-8.36 - 12.63)	-2.62, 4.82	0.551	14.85, 61.05 (25.00 - 54.11)

Table VI-2. Statistical Summary of Combined-Site Seed Nutrient Content for MON 88302 vs. the Conventional Control (continued)

Analytical Component (Units)[1]	MON 88302[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88302 minus Control) Mean (S.E.) (Range)	95% CI[3] Lower, Upper	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Mineral						
Phosphorus (g/100g dw)	0.72 (0.042) (0.56 - 0.87)	0.72 (0.041) (0.56 - 0.93)	-0.0090 (0.022) (-0.095 - 0.16)	-0.055, 0.037	0.692	0.38, 1.06 (0.44 - 0.87)
Potassium (g/100g dw)	0.64 (0.053) (0.48 - 0.90)	0.64 (0.052) (0.53 - 0.81)	0.0016 (0.025) (-0.097 - 0.14)	-0.056, 0.060	0.951	0.39, 0.96 (0.50 - 0.92)
Zinc (mg/kg dw)	35.58 (1.78) (29.81 - 45.56)	33.01 (1.76) (28.46 - 40.66)	2.57 (1.83) (-4.50 - 11.44)	-1.66, 6.80	0.198	20.19, 48.23 (22.18 - 47.61)
Vitamin (mg/100g dw)						
Vitamin E (α-tocopherol)	11.06 (2.08) (1.30 - 16.55)	8.85 (2.08) (3.33 - 11.77)	2.21 (1.66) (-6.92 - 8.09)	-1.61, 6.03	0.218	3.88, 17.28 (2.62 - 14.84)

[1]dw = dry weight; fw = fresh weight; FA = fatty acid.
[2]MON 88302 treated with glyphosate.
[3]Mean (S.E.) = least-square mean (standard error); CI – confidence interval.
[4]Control refers to the genetically similar, conventional control Ebony.
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial conventional reference varieties. Negative limits were set to zero.

Table VI-3. Statistical Summary of Combined-site Canola Seed Anti-nutrients for MON 88302 vs. Conventional Control

Analytical Component (Units)[1]	MON 88302[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88302 minus Control) Mean (S.E.) (Range)	95% CI[3] Lower, Upper	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Anti-nutrient						
Alkyl Glucosinolate (µmole/g dw)	3.68 (0.43) (1.19 - 5.87)	5.08 (0.42) (2.45 - 8.28)	-1.40 (0.59) (-6.11 - 1.43)	-2.69, -0.11	0.035	0, 29.02 (2.32 - 28.33)
Indolyl Glucosinolate (µmole/g dw)	3.50 (0.51) (0.49 - 5.76)	3.89 (0.50) (1.83 - 5.89)	-0.39 (0.45) (-3.05 - 2.83)	-1.42, 0.64	0.408	1.37, 6.62 (1.84 - 7.18)
Phytic Acid (% dw)	1.95 (0.18) (1.20 - 2.58)	2.11 (0.18) (1.46 - 2.77)	-0.16 (0.083) (-0.67 - 0.68)	-0.33, 0.010	0.064	0.70, 3.52 (1.10 - 2.71)
Sinapic Acid (% dw)	0.86 (0.12) (0.16 - 1.08)	0.88 (0.12) (0.65 - 0.97)	-0.023 (0.11) (-0.76 - 0.21)	-0.27, 0.22	0.837	0.57, 1.13 (0.48 - 0.99)
Total Glucosinolate (µmole/g dw)	7.35 (0.87) (1.73 - 11.42)	9.08 (0.86) (4.38 - 12.72)	-1.73 (1.01) (-9.21 - 3.58)	-4.06, 0.61	0.127	0, 32.20 (5.52 - 31.98)

[1]dw = dry weight.
[2]MON 88302 treated with glyphosate.
[3]Mean (S.E.) = least-square mean (standard error); CI = confidence interval.
[4]Control refers to the genetically similar, conventional control Ebony.
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial conventional reference varieties. Negative limits were set to zero.

Table VI-4. Literature Ranges for Components in Canola Seed

Component[1]	Literature Range[2]
Proximates (% dw)	
Ash	4.067 – 5.917[a]
Carbohydrates	N
Moisture (% fw)	3.177 – 8.045 [a]; 7.4 – 10.0[b]
Protein	21.30 – 28.125[a]; 18.7 – 26.0[b]; 17.4 – 23.0[c]; 21.1 – 26.7[d]
Total Fat	35.59 – 44.93[a]; 24.0 – 43.6[b]; 42.0 – 49.5[d]
Fiber (% dw)	
Acid Detergent Fiber (ADF)	11.934 – 26.799 [a];11.6[f]; 12.4[g]; 22.2[h]
Neutral Detergent Fiber (NDF)	18.653 – 34.720[a]; 17.8[f]; 16.49[g]; 31.3[h]
Total Dietary Fiber	N
Amino Acids (% dw)	
Alanine	0.93 – 0.96[b] ; 1.15 – 1.38[e]
Arginine	1.13 – 1.21 [b]; 2.23 – 2.46[e]
Aspartic acid	1.54 – 1.59[e]
Cystine/Cysteine	0.52 – 0.54 [b]
Glutamic acid	4.60 – 4.71[e]
Glycine	1.04 – 1.06 [b]; 2.20 – 2.22[e]
Histidine	0.51 – 0.66 [b]; 0.80 – 0.82[e]
Isoleucine	0.80 – 0.86 [b]; 0.96 – 1.03[e]
Leucine	1.35 – 1.47 [b]; 1.83 – 1.99[e]
Lysine	1.03 – 1.19 [b]; 1.67 – 1.85[e]
Methionine	0.42 – 0.44 [b]
Phenylalanine	0.75 – 0.82 [b]; 0.90 – 1.03[e]
Proline	1.19 – 1.33 [b]; 3.36 – 3.74[e]
Serine	0.90 – 0.94 [b]; 1.44 – 1.55[e]
Threonine	0.87 – 0.94 [b]; 1.28 – 1.30[e]
Tryptophan	0.23 – 0.27[b];
Tyrosine	0.51 – 0.59 [b]; 0.81 – 0.92[e]
Valine	1.02 – 1.13 [b]; 1.45 – 1.55[e]
Vitamins (mg/kg dw)	
Vitamin E (α-tocopherol)	71.1 – 108.4[i]

Table VI-4. Literature Ranges for Components in Canola Seed (continued)

Component[1]	Literature Range[2]
Minerals	
Calcium (% dw)	0.29 – 0.48[b]; 0.348 – 0.729[a]
Copper (mg/kg dw)	7[b]; 1.388 – 5.492[a]
Iron (mg/kg dw)	ND[b]; 0.0 – 965.6[a]
Magnesium (% dw)	0.29 – 0.31[b]; 0.272 – 0.402[a]
Manganese (mg/kg dw)	ND[b]; 33.813 – 64.757[a]
Phosphorus (% dw)	0.48 – 0.85[b]; 0.581 – 0.895[a]
Potassium (% dw)	0.83 – 0.91[b]; 0.681 – 1.016[a]
Sodium (% dw)	0.05[b]; 0.003 – 0.030[a]
Zinc (mg/kg dw)	62[b]; 0 – 126.953[a]
Fatty Acids (% total)	
16:0 Palmitic	3.3 – 6.0[b]
16:1 Palmitoleic	0.1 – 0.6[b]
18:0 Stearic	1.1 – 2.5[b]
18:1 Oleic	52.0 – 66.9[b]
18:2 Linoleic	16.1 – 24.8[b]
18:3 Linolenic	6.4 – 14.1[b]
20:0 Arachidic	0.2 – 0.8[b]
20:1 Eicosenoic	0.1 – 3.4[b]
20:2 Eicosadienoic	0.0 – 0.1[b]
20:3 Eicosatrienoic	N
20:4 Arachidonic	N
22:0 Behenic	0.0 – 0.5[b]
22:1 Erucic	0.0 – 2.0[b]
24:0 Lignoceric	0.0 – 0.2[b]
24:1 Nervonic	0.0 – 0.04[b]
Anti-nutrients	
Total Glucosinolates (µmol/g)	6 – 29[b]; 7.8 – 26.8[c]; 18 – 57[j]
Phytic Acid (% dw)	2.0 – 5.0[b]
Sinapine (% dw)	0.6 – 1.8[b]

[1]fw = fresh weight; dw = dry weight; dm = dry matter; ND defined as below the level of detection; N defined as not reported.
[2]Literature Range = Values published for low erucic acid oilseed rape (canola).
Citations = [a](Dairy One Forage Lab, 2010); [b](OECD, 2001); [c](Pritchard et al., 2000); [d](Barthet and Daun, 2005); [e](Wang et al., 1999); [f](NRC, 2001); [g](Mustafa et al., 2000); [h](Leupp et al., 2006); [i](Marwede et al., 2004);.[j](Mailer and Pratley, 1990).
Conversions: mg/100g dw × 10 = mg/kg dw; g/100g dw× 10 = mg/g dw.

VI.B. Compositional Assessment of MON 88302 Conclusion

Analyses of nutrient, toxicant, and anti-nutrient levels in MON 88302 and the conventional control were conducted to assess compositional equivalence. The tissue analyzed was seed harvested from plants grown at five field sites in the U.S. and Canada during the 2009 field season. The compositional analysis, conducted in accordance with OECD guidelines, included measurement of nutrient, toxicant, and anti-nutrient components in a genetically similar conventional control variety, Ebony and also in commercial conventional reference varieties that have a history of safe consumption to establish the natural range of variability. MON 88302, the conventional control, and commercial reference varieties were treated with conventional weed control programs. In addition, MON 88302 plots were treated with glyphosate herbicide at a target rate of 1.6 lb a.e./acre (1800 g a.e./ha).

The following components showed no significant differences in mean values between MON 88302 and the conventional control: proximates, ADF, NDF, amino acids, 16:0 palmitic acid, 20:1 eicosenoic acid, 24:0 lignoceric acid, and 24:1 nervonic acid, minerals, and vitamin E.

The significant differences ($\alpha = 0.05$) in nutrient and anti-nutrient (including toxicant) components were evaluated using considerations relevant to the safety and nutritional quality of MON 88302 when compared to the conventional control:

1) The relative magnitudes of differences for nutrients that were statistically significant in the combined-site analysis were small (4.52% to 20.01%), when considered relative to the natural variability determined by the 99% tolerance interval established by the concurrently grown commercial reference varieties that have a history of safe consumption. The relative magnitude of difference for the anti-nutrient alkyl glucosinolate that was statistically significant in the combined site analysis was small (27.59%) when considered relative to the natural variability determined by the 99% tolerance interval established by the concurrently grown commercial reference varieties that have a history of safe consumption.

2) Mean values for these nutrient and anti-nutrient components from the combined-site analysis of MON 88302 fell within the 99% tolerance interval established from the commercial reference varieties grown concurrently. Therefore, the differences were within the range of natural variability of those components in commercial canola varieties that have a history of safe consumption (Tables VI-2 and VI-3).

3) Assessment of the reproducibility of the combined-site differences at the five individual sites showed similar significant differences ($\alpha = 0.05$) at multiple sites. In all instances the individual site mean values for these components in MON 88302 were within the 99% tolerance interval established from the concurrently grown commercial reference varieties. Therefore, these components

were within the range of natural variability in commercial canola that have a history of safe consumption.

4) With the exception of TDF and alkyl glucosinolates, all of the components identified as significantly different from the conventional control were within the natural variability of these components in commercial canola as published in the scientific literature. There are no relevant published values for TDF and alkyl glucosinolates.

This analysis provides a comprehensive comparative assessment of the levels of key nutrients, toxicants, and anti-nutrients in seed of MON 88302 and the conventional control, discussed in the context of natural variability of commercial canola. Results of the comparison indicate that the seed of MON 88302 is compositionally equivalent to that of the conventional canola control. The genetic modification in MON 88302 does not meaningfully impact seed composition and therefore the food and feed safety and nutritional quality of this product is comparable to conventional canola that has a history of safe consumption.

Canola seed is processed to oil and meal. The processing of MON 88302 is not expected to be any different from that of conventional canola. As described in this section, detailed compositional analyses of key components of MON 88302 have been performed and have demonstrated that MON 88302 is compositionally equivalent to conventional canola. Additionally, the mode of action of CP4 EPSPS protein, as described in Section V.A., is well understood, and there is no reason to expect interactions with important nutrients or endogenous toxicants or anti-nutrients that may be present in canola. Therefore, when MON 88302 and its progeny are used on a commercial scale as a source of food or feed, these products are not expected to be different from the equivalent foods or feeds originating from conventional canola.

VII. PHENOTYPIC, AGRONOMIC, AND ENVIRONMENTAL INTERACTIONS ASSESSMENT

This section provides an assessment of the phenotypic, agronomic, and environmental interaction characteristics of MON 88302 compared to the conventional control Ebony. The data support a conclusion that MON 88302 is similar to conventional canola with the exception of the glyphosate-tolerance trait and, therefore, is no more likely to pose a plant pest risk or have a significant environmental impact. These conclusions are based on the results of multiple evaluations reported here.

Phenotypic, agronomic, and environmental interaction characteristics of MON 88302 were evaluated in a comparative manner to assess plant pest potential. These assessments included evaluation of seed germination characteristics, plant growth and development characteristics, observations for plant responses to abiotic stress, plant-disease and plant-arthropod interactions, and pollen characteristics. Results from the phenotypic, agronomic, and environmental interaction assessment demonstrate that MON 88302 does not possess a) increased weediness characteristics; b) increased susceptibility or tolerance to specific abiotic stress, diseases, or arthropods; or c) characteristics that would confer a plant pest risk or a significant environmental impact compared to the conventional control.

VII.A. Characteristics Measured for Assessment

In the phenotypic, agronomic, and environmental interactions assessment of MON 88302, data were collected to evaluate specific aspects of altered plant pest potential. A detailed description of the regulated article phenotype is requested as part of the petition for determination of nonregulated status in 7 CFR § 340. 6 including differences from the unmodified recipient organism that would "substantiate that the regulated article is unlikely to pose a greater plant pest risk than the unmodified organism from which it was derived…" As part of the characterization of MON 88302, data were collected to provide a detailed phenotypic, agronomic, and environmental interaction description of MON 88302. A subset of these data were included in an evaluation of specific characteristics related to altered plant pest potential.

As part of the phenotype description, the plant characterization of MON 88302 encompassed five general categories: 1) germination, dormancy, and emergence; 2) vegetative growth; 3) reproductive growth (including pollen characteristics); 4) seed retention on the plant and lodging; and 5) plant response to abiotic stress and interactions with diseases and arthropods. An overview of the characteristics assessed is presented in Table VII-1.

The phenotypic, agronomic, and environmental interactions data were evaluated from a basis of familiarity (OECD, 1993) and were comprised of a combination of field and laboratory studies conducted by scientists who are familiar with the production and evaluation of canola. In each of these assessments, MON 88302 was compared to a conventional control that had a genetic background similar to MON 88302 but did not contain the glyphosate-tolerance trait. In addition, multiple commercial canola varieties,

(see Appendix F, G, and H and Tables F-1, G-1, and H-1) were included to provide a range of comparative values that are representative of commercial canola varieties for each measured phenotypic, agronomic, and environmental interaction characteristic. Reference varieties used in these studies were selected to represent a range of genetic backgrounds and phenotypic characteristics and have been grown in the canola production regions of Canada and the U.S. The commercial reference varieties reflect a range of values for the agronomic, phenotypic and environmental interaction characteristics assessed within the crop and therefore can provide context for interpreting experimental results.

Table VII-1. Phenotypic, Agronomic and Environmental Interaction Characteristics Evaluated in U.S. and Canadian Field Trials or Laboratory Studies

Data category	Characteristics measured (associated section where discussed)	Evaluation timing	Evaluation description
Seed germination, dormancy, and emergence	Normal germinated (VII.C.1)	Day 7 (15/25 °C)	Percentage of seed producing seedlings exhibiting normal developmental characteristics[2]
	Abnormal germinated (VII.C.1)	Day 7 (15/25 °C)	Percentage of seed producing seedlings that could not be classified as normal germinated[2]
	Germinated (VII.C.1)	Day 7 and 14 (5, 15, 25, 30, and 5/25 °C)	Percentage of seed that had germinated normally or abnormally
	Dead (VII.C.1)	Day 7 and 14 (15/25 °C); Day 7, 14 and 21 (5, 15, 25, 30, and 5/25 °C)	Percentage of seed that had visibly deteriorated and become soft to the touch or did not germinate and was determined to be non-viable by tetrazolium test[2]
	Viable non-dormant (VII.C.1)	Day 21 (5, 15, 25, 30, and 5/25 °C)	Percentage of seed that did not germinate at sub-optimal temperatures but when subsequently moved to the AOSA-recommended temperature (15/25°C) they did germinate[2]
	Dormant (VII.C.1)	Day 14 (15/25 °C); Day 21 (5, 15, 25, 30, and 5/25 °C)	Percentage of seed that did not germinate when incubated at any of the tested temperatures (viability determined by a tetrazolium test[2])
	Early stand count (VII.C.2.1)	Seedling (G.S. 12 – 14)[1]	Number of emerged plants estimated by counting plants from three non-systematically chosen linear meter rows per plot
	Final stand count (VII.C.2.1)	Post-harvest	Number of plants at harvest estimated by counting the stems after harvest from three non-systematically chosen linear meter rows per plot
Vegetative growth	Seedling vigor (VII.C.2.1)	Seedling (G.S. 12 – 14)[1]	Rated on a 1-9 scale, where 1 = excellent and 9 = poor vigor
	Plant height (VII.C.2.1)	Maturity (G.S. 71 – 89)[1]	Distance (inches) from the soil surface to the top of the main raceme of 15 representative plants per plot
Reproductive growth	Days to 50% flowering (VII.C.2.1)	Flowering (G.S. 60 – 69)[1]	Number of days after planting when approximately 50% of the plants in each plot had one or more flowers
	Seed maturity (VII.C.2.1)	Maturity (G.S. 80 – 89)[1]	Number of days after planting when 30% or more of the seed in the lower 1/3 of the main raceme had changed from a green color to black/brown/tan color
	Pollen viability (VII.C.3)	Flowering	Percentage of viable pollen based on pollen grain staining characteristics
	Pollen morphology (VII.C.3)	Flowering	Diameter (μm) of viable pollen grains
	Seed moisture (VII.C.2.1)	Harvest	Percent moisture content of harvested seed
	Seed quality (VII.C.2.1)	Harvest	Percentage of distinctly green seeds from a 100 seed subsample of the harvested seed
	Yield (VII.C.2.1)	Harvest	Bushels of harvested seed per acre, adjusted to 8% moisture

Table VII-1. Phenotypic, Agronomic and Environmental Interaction Characteristics Evaluated in U.S. and Canadian Field Trials or Laboratory Studies (continued)

Data category	Characteristics measured (associated section where discussed)	Evaluation timing	Evaluation description
Seed retention and lodging	Lodging (VII.C.2.1)	Maturity (G.S. 80 – 89)[1]	Rated on 1 - 9 scale, where 1 = completely upright and 9 = completely flat or lodged
	Quantitative pod shattering (VII.C.2.1)	Maturity (G.S. 83 – 89)[1]	A collection tray placed in the plant canopy of each plot and seed losses from shattering counted once per week for three weeks
	Visual pod shattering (VII.C.2.1	Maturity (G.S. 83 – 89)[1]	A rating scale of 1 - 9 scale, where 1 = 0 to 10% shatter, and each subsequent value on the scale increasing in 10% increments up to 9 = greater than 80% shatter.
Plant-environment interactions	Plant response to abiotic stress (VII.C.2.2)	Four times during growing season	Qualitative assessment of each plot, with rating on a 0 - 9 scale, where 0 = no symptoms and 9 = severe symptoms
	Disease damage (VII.C.2.2)	Four times during growing season	Qualitative assessment of each plot, with rating on a 0 - 9 scale, where 0 = no symptoms and 9 = severe symptoms
	Arthropod-related damage (VII.C.2.2)	Four times during growing season	Qualitative assessment of each plot, with rating on a 0 - 9 scale, where 0 = no symptoms and 9 = severe symptoms
	Flea beetle damage (VII.C.2.2)	Two times from seedlings (G.S. 12 - 16)[1]	Damage was assessed quantitatively from 10 plants per plot using a 0 - 10 rating scale where 0 = no damage and 10 = 100% of leaf area damaged
	Seedpod weevil damage (VII.C.2.2)	Ripening (G.S. 80 – 89)[1]	Damage was assessed quantitatively from 10 plants (5 pods per plant) by counting the number of exit holes in each of 50 pods per plot
	Arthropod abundance (VII.C.2.2)	Four times during growing season	Identification and enumeration of pest and beneficial arthropods abundance in cone beat sheet samples

[1]Canola plant growth stages (G.S.) were determined using descriptions and guidelines outlined in the Canola Council of Canada Growers Manual (Thomas, 2003a): 12 = two true leaves unfolded; 14 = four true leaves unfolded; 16 = six true leaves unfolded; 60 – 69 = flowering; 70 -79 = pods begin to develop to nearly all pods have reached final size; 80 = ripening begins (seed green, filling pod cavity); 81 = 10% of pods ripe, seeds black and hard; 83 = 30% of seeds have changed color; 89 = fully ripe, nearly all seeds have changed color.

[2]Methods for testing seed were consistent with AOSA guidelines (AOSA, 2009a; 2009b).

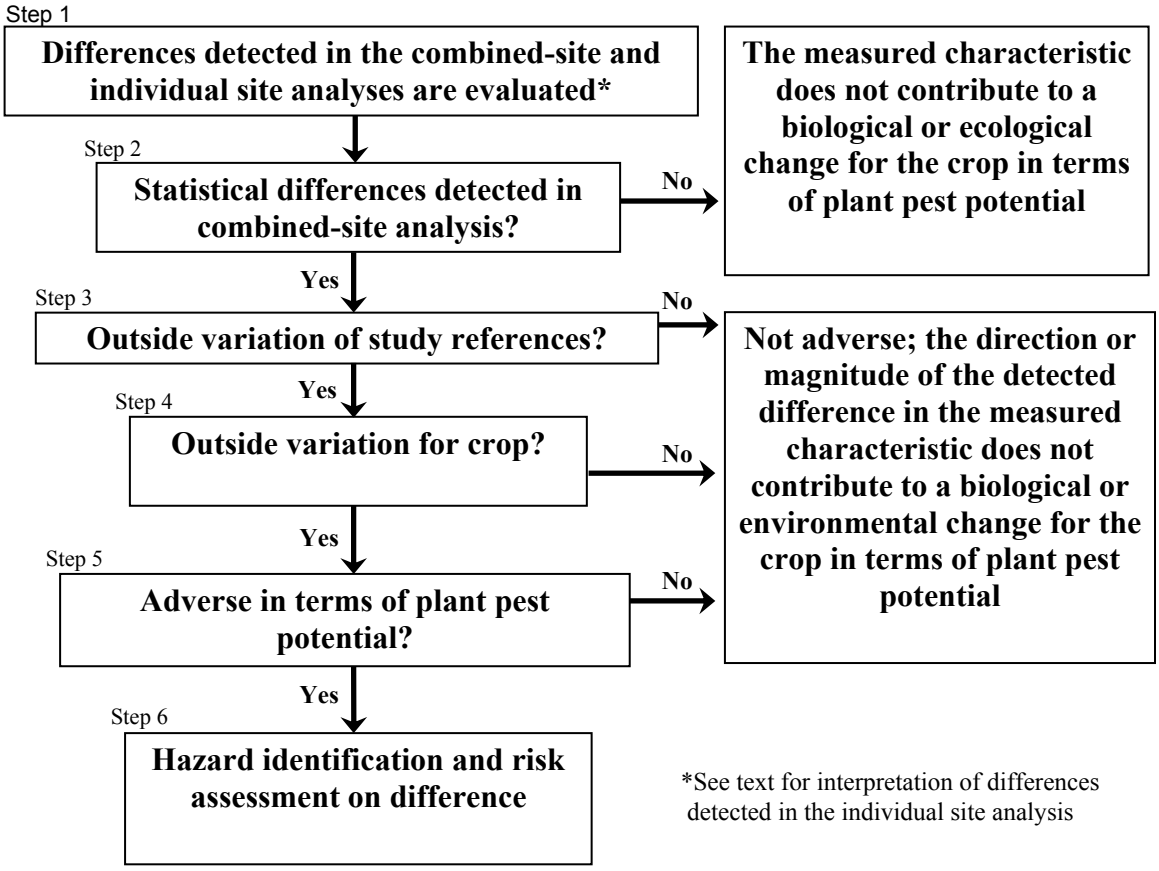
VII.B. Interpretation of Phenotypic and Environmental Interaction Data

Plant pest risk assessments for biotechnology-derived crops are comparative assessments. Familiarity provides a basis from which the potential environmental impact of a biotechnology-derived plant can be evaluated. The concept of familiarity is based on the fact that the biotechnology-derived plant is developed from a well-characterized conventional plant variety. Familiarity considers the biology of the crop, the introduced trait, the receiving environment and the interaction of these factors, and provides a basis for comparative environmental risk assessment between a biotechnology-derived plant and its conventional counterpart.

Expert knowledge and experience with conventionally bred canola was the basis for selecting appropriate endpoints and estimating the range of responses that would be considered typical for canola. As such, MON 88302 was compared to the conventional control in the assessment of phenotypic, agronomic, and environmental interaction characteristics. An overview of the characteristics assessed is presented in Table VII-1. A subset of the data relating to well-understood weediness criteria (Baker, 1974) (*e.g.,* seed dormancy, pre-harvest seed loss characteristics, lodging) was used to assess whether there was an increase in weediness potential of MON 88302, an element of Animal and Plant Health Inspection Service's (APHIS) plant pest determination. Evaluation of environmental interaction characteristics (*e.g.,* plant abiotic stress, plant-disease, and plant-arthropod interactions) was also considered in the plant pest risk assessment. Based on all of the data collected, an assessment was made to determine if MON 88302 is likely to pose an increased plant pest risk compared to conventional canola. Prior to analysis, the overall dataset was evaluated for evidence of biologically relevant changes, and for possible evidence of an unexpected plant response. No unexpected observations or issues were identified.

VII.B.1. Interpretation of Detected Differences Criteria

Comparative plant characterization data between a biotechnology-derived crop and the conventional control are interpreted in the context of contributions to increased plant pest potential as assessed by APHIS. Under the framework of familiarity, characteristics for which no differences are detected support a conclusion of no increased pest potential of the biotechnology-derived crop compared to the conventional crop. Characteristics for which differences are detected are considered in the step-wise method described below. All detected differences for a characteristic are considered in the context of whether or not the difference would increase the plant pest potential of the biotechnology-derived crop. Ultimately, a weight of evidence approach considering all characteristics and studies was used for the final risk assessment of differences and their significance. Figure VII-1 illustrates the step-wise assessment process employed in detail:



Note: A "no" answer at any step indicates that the characteristic does not contribute to a biological or environmental change for the crop in terms of plant pest potential and subsequent steps are not considered. The subsequent step is considered if the answer is "yes" or uncertain. It should be noted that some steps are not necessarily applicable for all data. For example, some data are not statistically analyzed (*e.g.,* abiotic stress response, disease damage data) or are only statistically analyzed within individual sites (*e.g.,* arthropod abundance data); however, the method for interpreting these data follows the same general approach.

Figure VII-1. Schematic Diagram of Agronomic and Phenotypic Data Interpretation Methods

Steps 1 and 2 - Evaluate Detected Statistically Significant Differences

Data on each measured characteristic are statistically analyzed, where appropriate, within each individual site and in a combined-site analysis, in which data are pooled among sites. Differences detected in the individual site analysis must be observed in the combined-site analysis to be considered further for plant pest potential. Any difference detected in the combined-site analysis is assessed further.

Step 3 - Evaluate Differences Relative to Range of Commercial Reference Varieties

If a difference for a characteristic is detected in the combined-site analysis across multiple environments or when only an individual site analysis is conducted and a

difference for a characteristic is detected, then the mean value for the biotechnology-derived crop is assessed relative to the commercial reference varieties.

Step 4 - Evaluate Differences in the Context of the Crop

If the mean value of the biotechnology-derived crop is outside the variation of the commercial reference varieties (*e.g.,* reference range), the mean value of the biotechnology-derived crop for the characteristic is assessed relative to known values common for the crop (*e.g*., published values).

Step 5 - Plant Pest Potential

If the mean value of the biotechnology-derived crop is outside the range of values common for the crop, the detected difference is then assessed for whether or not it is adverse in terms of plant pest potential.

Step 6 - Conduct Risk Assessment on Identified Hazard

If an adverse effect (hazard) is identified, a risk assessment on the difference is conducted. The risk assessment considers contributions to enhanced plant pest potential of the crop itself, the impact of differences detected in other measured characteristics, and potential for, and effects of trait transfer to feral populations of the crop or a sexually compatible species.

VII.C. Comparative Assessments of the Phenotypic, Agronomic, and Environmental Interaction Characteristics of MON 88302

This section provides the results of comparative assessments conducted in replicated laboratory and/or multi-site field experiments to provide a detailed phenotypic, agronomic, and environmental interaction description of MON 88302. The characteristics of MON 88302 evaluated in these assessments included: seed dormancy and germination characteristics (Section VII.C.1), plant phenotypic, agronomic, and environmental interaction characteristics under field conditions (Section VII.C.2), and pollen characteristics (Section VII.C.3). Additional details for each assessment are provided in Appendices F, G, and H.

VII.C.1. Seed Dormancy and Germination Characteristics

USDA-APHIS considers the potential for weediness to constitute a plant pest risk (7 CFR § 340.6). Seed germination and dormancy mechanisms vary with species and their genetic basis tends to be complex. Seed dormancy is an important characteristic that is often associated with plants that are considered weeds (Anderson, 1996; Lingenfelter and Hartwig, 2007). Canola seed dormancy is known to occur under field conditions and can contribute to seed persistence in soil (Gulden et al., 2004) and Section IX.C). Standardized germination assays are routinely used to measure the germination characteristics of canola seed. The Association of Official Seed Analysts (AOSA), an internationally recognized seed testing organization, recommends a temperature regime of 15/25 °C for testing the germination characteristics of canola seed (AOSA, 2009a).

Comparative assessments of seed dormancy and germination characteristics were conducted on MON 88302 and the conventional control. In addition, four commercial reference varieties were included to provide a range of comparative values that are representative of commercial canola varieties. The seed lots for MON 88302, conventional control, and the commercial reference varieties were produced under field conditions in Grand Forks County, ND in 2009, a geographic area which represents an environment with conditions suitable for canola production. In addition to the AOSA recommended temperature regime of 15/25 °C, seed was tested at five additional temperature regimes of constant 5, 15, 25 or 30 °C, and alternating 5/25 °C to assess seed germination properties. The details of the experimental materials and methods are presented in Table VII-1 and Appendix F.

No statistically significant differences ($\alpha = 0.05$) were detected between MON 88302 and the control at any of the temperature regimes for normal and abnormal germination and dead seed (Table VII-2). No statistically significant differences ($\alpha = 0.05$) were detected between MON 88302 and the control at the 5 ºC temperature regime for viable non-dormant seed (defined in Table VII-1; Table VII-2). Analysis of variance was not conducted on dormant seed in all temperatures and viable non-dormant seed (defined in Table VII-1) seed at the 15 ºC, 25 ºC, 30 ºC and 5/25 ºC temperature regimes due to low numbers of seed in these categories.

Although statistical comparisons were not made due to a low number of dormant seed in the AOSA-recommended temperature regime (15/25 ºC), MON 88302 numerically had fewer dormant seeds than the conventional control (0.0% vs. 0.3%). A reduction in the number of dormant seed would not increase plant weediness since non-dormant seed would be more likely to germinate reducing the potential for persistence in the soil seed bank (Gulden et al., 2003). Although, no statistical comparisons were made for viable non-dormant seed in the 15 ºC, 25 ºC, 30 ºC and 5/25 ºC temperature regimes, the magnitude of the difference for seed of MON 88302 and the conventional control was small and there were no observable trends in the mean difference in this category across the different temperature regimes.

The biological characteristics evaluated were used to characterize MON 88302 in the context of plant pest risk assessment. Based on the assessed characteristics, the results of this study demonstrate that there were no changes in the dormancy or germination characteristics that are indicative of increased plant weediness or pest potential of MON 88302 compared to the conventional control.

Table VII-2. Germination Characteristics of MON 88302 and Conventional Control

Temperature Regime (°C)	Germination[1] Category	Mean% (S.E.)[2] MON 88302	Control	Reference Range[3]
5	Germinated	95.3 (1.0)	96.5 (0.6)	99.0-99.8
	Dead	2.3 (0.3)	1.8 (0.9)	0.0-0.5
	Viable non-dormant	2.5 (1.0)	1.8 (0.9)	0.3-0.5
	Dormant †	0.0 (0.0)	0.0 (0.0)	0.0-0.0
15	Germinated	97.5 (1.4)	99.0 (0.4)	98.5-100.0
	Dead	2.5 (1.4)	0.8 (0.3)	0.0-1.5
	Viable non-dormant †	0.0 (0.0)	0.3 (0.3)	0.0-0.0
	Dormant †	0.0 (0.0)	0.0 (0.0)	0.0-0.0
25	Germinated	98.5 (0.6)	98.8 (0.6)	98.5-99.8
	Dead	1.3 (0.5)	1.0 (0.7)	0.3-1.5
	Viable non-dormant †	0.3 (0.3)	0.3 (0.3)	0.0-0.0
	Dormant †	0.0 (0.0)	0.0 (0.0)	0.0-0.0
30	Germinated	98.8 (0.6)	97.3 (0.9)	97.0-99.5
	Dead	1.0 (0.7)	2.8 (0.9)	0.5-2.8
	Viable non-dormant †	0.3 (0.3)	0.0 (0.0)	0.0-0.3
	Dormant †	0.0 (0.0)	0.0 (0.0)	0.0-0.0
5/25	Germinated	99.3 (0.5)	99.0 (0.4)	96.8-99.5
	Dead	0.8 (0.5)	1.0 (0.4)	0.5-3.0
	Viable non-dormant †	0.0 (0.0)	0.0 (0.0)	0.0-0.3
	Dormant †	0.0 (0.0)	0.0 (0.0)	0.0-0.0
15/25 (AOSA)	Normal germinated	98.0 (1.1)	98.0 (0.7)	96.3-98.5
	Abnormal germinated	1.3 (1.0)	1.3 (0.3)	1.5-3.8
	Dead	0.8 (0.5)	0.5 (0.5)	0.0-1.3
	Dormant †	0.0 (0.0)	0.3 (0.3)	0.0-0.0

Note: experimental design was a randomized complete block with four replicates (n = 4).
No statistically significant differences were detected (α = 0.05) between MON 88302 and the conventional control.
[1]Categories evaluated were modified from Association of Official Seed Analysts (AOSA, 2007; 2009a; 2009b). Further details and definitions are provided in Table F-2.
[2]In some instances, the total percentage for both MON 88302 and the conventional control did not equal 100% due to numerical rounding of the means.
[3]Minimum and maximum means determined from among the commercial reference varieties.
†Analysis of variance (ANOVA) was not conducted due to low numbers of seed within these categories.

VII.C.2. Field Phenotypic, Agronomic, and Environmental Interactions Characteristics

Phenotypic, agronomic, and environmental interactions characteristics were evaluated under field conditions as part of the plant characterization assessment of MON 88302. These data were developed to provide USDA-APHIS with a detailed description of MON 88302 relative to the conventional control and commercial reference varieties. According to 7 CFR § 340.6, as part of the petition to seek deregulation, a petitioner must submit "a detailed description of the phenotype of the regulated article." This information is being provided to assess whether there are phenotypic differences between MON 88302 and the conventional control that may impact its pest potential. Certain growth, reproduction, and pre-harvest seed loss characteristics (*e.g.,* lodging, pod shattering) were used to assess whether there is an increase in weediness of MON 88302, an element of APHIS's plant pest risk determination. Environmental interactions were also assessed as an indirect indicator of phenotypic changes to MON 88302 compared to the same comparators described above and are also considered in the plant pest risk assessment.

Data were collected from field trials located at eight field sites in the U.S. and nine field sites in Canada during 2009 to evaluate phenotypic, agronomic, and environmental interaction characteristics (Table VII-3). These 17 field sites provided a diverse range of environmental and agronomic conditions representative of commercial canola production areas in North America. The sites were planted in a randomized complete block design with four replicates per site. All plots of MON 88302, the conventional control, and the commercial reference varieties at each site were uniformly managed in order to assess whether the introduction of the glyphosate-tolerance trait altered the phenotypic and agronomic characteristics or the environmental interactions of MON 88302 compared to the conventional control. Glyphosate herbicide was not applied to any of the plots in the trial. A description of the evaluated phenotypic and environmental interaction characteristics and the designated developmental stages when evaluations occurred are listed in Table VII-1. The methods and detailed results of the individual site data comparisons are presented and discussed in Appendix G, while the combined-site analyses are summarized below. The results of this assessment demonstrated that the introduction of the glyphosate-tolerance trait did not alter MON 88302 compared to the conventional control in terms of weediness potential. The lack of differences in plant response to abiotic stress, disease damage, arthropod-related damage, and pest and beneficial arthropod abundance further support the conclusion that the introduction of the glyphosate-tolerance trait is not likely to result in increased plant pest potential or an adverse environmental impact from MON 88302 compared to conventional canola.

VII.C.2.1. Field Phenotypic and Agronomic Characteristics

A total of 12 phenotypic and agronomic characteristics were evaluated (Table VII-4 and Table G-4 of Appendix G). In the combined-site analysis in which the data were pooled among the sites, no statistically significant differences were detected ($\alpha = 0.05$) between MON 88302 and the conventional control for early stand count, seedling vigor, seed maturity, lodging, plant height, visual rating for pod shattering, quantitative pod

shattering, seed quality, yield, and final stand count (Table VII-4). Two statistically significant differences were detected between MON 88302 and the conventional control in the combined-site analysis. MON 88302 reached first flowering later than the conventional control (61.1 vs. 56.2 days). However, the mean value of MON 88302 for days to first flowering was within the natural variability of the commercial reference varieties (45.9 – 67.5 days). Therefore, the difference in days to first flower is unlikely to be biologically meaningful in terms of increased weediness potential. MON 88302 also had higher harvested seed moisture than the conventional control (13.2% vs. 11.7%). However, the mean value of MON 88302 for harvested seed moisture was within the natural variability of the commercial reference varieties (7.5% – 14.8%). Therefore, the difference in seed moisture is unlikely to be biologically meaningful in terms of increased weediness potential.

The plant phenotypic and agronomic characteristics and environmental interactions evaluated in this study were used to characterize the plant and its interactions with the environment and to assess the plant pest or weed potential of MON 88302 compared to the conventional canola control. Based on the assessed characteristics, the results of this study demonstrate that there were no unexpected changes indicative of increased plant pest potential or adverse environmental impact of MON 88302 compared to conventional canola.

Table VII-3. Field Phenotypic Evaluation Sites for MON 88302 during 2009

Location (County or RM, State or Province)[1]	Country	Location Code	USDA-APHIS Notification/CFIA Authorization Number
Leduc, AB	Canada	ABLE	09-MON1-378-CAN
Brookings, SD	U.S.	IARL	09-048-105n
Elton, MB	Canada	MBBR	09-MON1-378-CAN
Whitewater, MB	Canada	MBMI	09-MON1-378-CAN
Portage le Prairie, MB	Canada	MBNW	09-MON1-378-CAN
Otter Tail, MN	U.S.	MNCA	09-048-105n
Stearns, MN	U.S.	MNPY	09-048-105n
Wilkins, MN	U.S.	MNRO	09-048-105n
Grand Forks, ND	U.S.	NDBI	09-069-101n
McHenry, ND	U.S.	NDBO	09-044-107n
Brookings, SD	U.S.	NDCL	09-048-105n
McHenry, ND	U.S.	NDVA	09-044-107n
Flett's Springs, SK	Canada	SKME	09-MON1-378-CAN
Corman Park, SK	Canada	SKRA	09-MON1-378-CAN
Wallace, SK	Canada	SKRO	09-MON1-378-CAN
Rosthern, SK	Canada	SKSA	09-MON1-378-CAN
Viscount, SK	Canada	SKWA	09-MON1-378-CAN

[1] RM = rural municipality, AB Alberta. SD South Dakota, MB Manitoba, MN Minnesota, ND North Dakota, SK Saskatchewan.

Table VII-4. Combined-Site Comparison of MON 83302 to Conventional Control During 2009 for Phenotypic and Agronomic Characteristics

Phenotypic Characteristic (units)	MON 88302 Mean (S.E.)	Control Mean (S.E.)	Reference Range[1] Minimum	Reference Range[1] Maximum
Early stand count (# plants per linear meter)[2]	18.2 (0.8)	19.2 (0.7)	12.7	30.1
Seedling vigor (1-9 scale)[3]	5.1(0.2)	4.5 (0.2)	1.9	7.0
Days to first flowering (Days after planting)[4]	61.1 (1.5)*	56.2 (1.4)	45.9	67.5
Seed maturity (Days after planting)[5]	102.6 (1.4)	101.3 (1.4)	84.0	108.0
Lodging (1-9 scale)[6]	1.7 (0.3)	1.8 (0.2)	1.0	4.6
Plant height (inches)[7]	45.4 (0.9)	44.3 (0.8)	29.5	47.5
Visual rating for pod shattering (0-9 visual scale)[8]	1.2 (0.1)	1.4 (0.1)	1.0	5.8
Quantitative pod shattering (seeds per ft.^{2})[9]	64.7 (22.7)	132.5 (52.2)	13.5	590.1
Seed moisture (%)[10]	13.2 (0.5)*	11.7 (0.4)	7.5	14.8
Seed quality (%)[11]	3.3 (0.5)	2.8 (0.5)	0.0	7.8
Yield (bushels per acre)[12]	43.4 (2.2)	44.5 (2.2)	11.7	72.3
Final stand count (# plants per linear meter)[13]	18.3 (0.7)	18.1 (0.6)	12.5	28.7

Note: The experimental design was a randomized complete block with four replicates. S.E. = Standard Error. The number of plots (n) used in the statistical analysis was 68 except where noted.

*Indicates a statistically significant difference between MON 88302 and the conventional canola control ($\alpha = 0.05$).

[1]Reference range = Minimum and maximum mean values among the 24 commercial canola reference varieties.

[2] Early stand count data plot number (n)=68 for MON 88302 and n=67 for the conventional control.

[3]Rated on a scale of 1-9, where 1 =excellent vigor, and 9 = poor vigor. Plot number (n)=48 for MON 88302 and the conventional control.

[4]Days to first flowering was determined from the number of days after planting when 50% of the plants in a plot had one or more flowers. Plot number (n)=58 for MON 88302 and n=59 for the conventional control.

[5]Seed maturity was determined as the number of days after planting when 30% or more of the seed in the lower 1/3 of the main raceme had changed from a green to black/brown/tan color. Plot number (n)=41 for MON 88302 and n=43 for the conventional control.

[6]Rated on a 0 - 9 scale, where 0 = completely upright plants and 9 = completely flat. Plot number (n)=64 for MON 88302 and the conventional control.

[7]Plant height data plot number (n)=60 for MON 88302 and the conventional control.

[8]Visual pod shattering was estimated with a rated on a 1 - 9 scale, where 1 = 0 to 10% shatter, and each subsequent value on the scale increasing in 10% increments up to 9 = greater than 80% shatter. Plot number (n)=56 for MON 88302 and the conventional control.
[9]Collection trays placed within the crop canopy and seed losses from shattering counted once per week for three weeks. Plot number (n)=12 for MON 88302 and the conventional control.
[10]Seed moisture data plot number (n)=57 for MON 88302 and n=60 for the conventional control.
[11]Seed quality was determined at harvest by counting the percentage of green seeds from a 100 seed subsample from each plot. Plot number (n)=64 for MON 88302 and the conventional control.
[12]Yield data plot number (n)=61 for MON 88302 and n=64 for the conventional control.
[13]Final stand counts plot number (n)=64 for MON 88302 and the conventional control.

VII.C.2.2. Environmental Interaction Characteristics

To determine the potential for increased plant pest characteristics, USDA-APHIS considers the environmental interaction of the biotechnology-derived crop compared to its conventional counterpart. Evaluations of environmental interactions were conducted as part of the plant characterization for MON 88302. In the 2009 North American field trials conducted for evaluation of phenotypic and agronomic characteristics of MON 88302, data were also collected on plant response to abiotic stress (drought, wind, nutrient deficiency, etc.), disease damage, arthropod damage, and arthropod abundance (Tables VII-5 and VII-6; Appendix G; Tables G-5, G-6, G-7, G-8, G-9 and G-10, respectively). These data were used as part of the environmental consequences (Section IX) to assess plant pest potential and provide an indication of potential effects of MON 88302 on non-target organisms (NTOs) as well as threatened and endangered species compared to the conventional control. In addition, multiple commercial reference varieties were included in the analysis to establish a range of natural variability for each assessed characteristic. The results of the field evaluations showed that the glyphosate-tolerance trait did not unexpectedly alter the assessed environmental interactions of MON 88302 compared to the conventional control. The lack of significant biologically-meaningful differences in plant response to abiotic stress, disease damage, arthropod damage, and pest and beneficial arthropod abundance support the conclusion that the introduction of the glyphosate-tolerance trait in MON 88302 is unlikely to result in increased plant pest potential or cause an adverse environmental impact compared to conventional canola.

In the 2009 field trials, the qualitative observations of plant response to abiotic stress, disease damage, and arthropod-related damage were performed four times during the growing season at all 17 sites. At four of the 17 sites (MBMI, MNPY, NDBI and SKSA) quantitative assessments were conducted four times for arthropod abundance, twice for flea beetle damage and once for seedpod weevil damage during the growing season.

In a qualitative assessment of plant response to abiotic stress, no differences were observed between MON 88302 and the conventional control for 130 out of 131 comparisons involving 9 assessed abiotic stressors (Table VII-5; Appendix G; Table G-5). One difference was observed in abiotic stress response in Observation 1 for frost damage at the MBBR site (where MON 88302 was severe and the conventional control was moderate). However, the observed frost damage to MON 88302 was within the range of the damage observed among the commercial reference varieties (slight – severe). In addition, the difference was not observed during any of the other 12 frost damage observations among the sites. Thus, the difference in frost damage rating during the single observation was not indicative of a consistent response associated with the trait and was considered not biologically meaningful in terms of plant pest potential or an adverse environmental impact from MON 88302 compared to conventional canola.

In a qualitative assessment of plant response to disease damage and arthropod damage, no differences were observed between MON 88302 and the conventional control for any of the 141 comparisons involving 16 assessed diseases or for any of the 165 comparisons for

any of the 13 assessed arthropods among all observations at the sites (Table VII-5, Appendix G; Tables G-6 and G-7).

In the combined-site analysis for a quantitative assessment of flea beetle (Chrysomelidae) damage and seedpod weevil (*Ceutorhynchus obstrictus*) damage, no statistically significant differences were detected ($\alpha = 0.05$) between MON 88302 and the conventional control from the four sites evaluated (Table VII-6).

In a quantitative assessment of pest and beneficial arthropod abundance, no statistically significant differences ($\alpha = 0.05$) were detected between MON 88302 and the conventional control for any of the 51 comparisons, including 36 arthropod pest comparisons and 15 beneficial arthropod comparisons, among the observations at the four sites (Table VII-5; Appendix G; Tables G-9 andG-10).

The results of the field evaluations showed that the glyphosate-tolerance trait did not unexpectedly alter the assessed environmental interactions of MON 88302 compared to the conventional control. The lack of significant biological differences in plant responses to abiotic stress, disease damage, arthropod-related damage, and pest and beneficial arthropod abundance support the conclusion that the introduction of the glyphosate-tolerance trait in MON 88302 is unlikely to result in increased plant pest potential or an adverse environmental impact as compared to conventional canola.

Table VII-5. Summary of Qualitative and Quantitative Environmental Interaction Assessments During 2009

Assessments[1]	Number of sites	Number of observations across sites	Number of observations where no differences were detected between MON 88302 and the conventional control
Qualitative			
Plant response to abiotic stress[2]	17	131	130
Disease damage	17	141	141
Arthropod damage	17	165	165
Quantitative			
Pest arthropod abundance	4	36	36
Beneficial arthropod abundance	4	15	15

[1]For each qualitative assessment, MON 88302 was considered different from the conventional control if the severity of injury to MON 88302 did not overlap with the severity of injury to the conventional control across all four replicates. Quantitative assessments were statistically analyzed ($\alpha = 0.05$).
[2]A single difference was observed for frost damage between MON 88302 and the conventional control during Observation 1 at the MBBR site: MON 88302 = severe, Conventional Control = moderate, Reference range = slight-severe.

Table VII-6. Combined-Site Analysis: Quantitative Assessment of Flea Beetle and Seedpod Weevil Damage to MON 88302 Compared to the Conventional Control in 2009 Field Trials[1]

Arthropod Pest	Damage assessment	Mean (S.E)		
		MON 88302	Control	Reference range[2]
Flea beetles[3] (Chrysomelidae)	Mean (S.E.) damage of 10 plants per plot (0-10 rating scale) – Observation 1	1.18 (0.24)	1.13 (0.24)	0.33 – 2.20
	Mean (S.E.) damage of 10 plants per plot (0-10 rating scale) – Observation 2	1.22 (0.19)	1.23 (0.20)	0.15 – 1.90
Seedpod weevil[4] (*Ceutorhynchus obstrictus*)	Mean (S.E.) number of holes in pods from 10 plants per plot	0.03 (0.02)	0.05 (0.02)	0.00 – 0.02

No statistically significant differences were detected between MON 88302 and the conventional control ($\alpha = 0.05$).
Note: The experimental design was a randomized complete block with four replicates. S.E. = Standard Error. The number of plots (n) used in the statistical analysis was 16 except where noted.
[1]Sites are as follows: MBMI = Whitewater, MB; MNPY = Stearns, MN; NDBI = Grand Forks, ND; SKSA = Rosthern, SK.
[2]Reference range = minimum and maximum mean values among the commercial reference varieties.
[3]Damage assessments for flea beetle were conducted 1-2 times during growing season. The first assessment was conducted during two to three weeks following crop emergence at all four sites. The second assessment was conducted approximately 1 week later at the MBMI, MNPY, and NDBI sites. Damage was assessed quantitatively using a 0 - 10 rating scale where 0 = no damage and 10 = 100% of leaf area damaged. The first flea beetle assessment at SKSA could not be conducted due to a severe rain storm (n = 12 plots).
[4]Damage assessments for seedpod weevil were conducted at the ripening stage.

VII.C.3. Pollen Characteristics

To determine the potential for MON 88302 to impact weediness of the receiving species, the USDA-APHIS considers the potential for gene flow and introgression of the biotechnology derived trait into other canola varieties and wild relatives. An assessment of pollen morphology and viability provides information to characterize the plant and are also pertinent to assessments of gene flow and, therefore, were assessed for MON 88302. In addition, characterization of pollen produced by MON 88302 and the conventional control is relevant to the plant pest risk assessment because it adds to the detailed description of the phenotype of MON 88302 compared to the conventional control.

The purpose of this evaluation was to assess the morphology and viability of pollen collected from MON 88302 compared to that of the conventional control. Pollen was collected from non-glyphosate-treated MON 88302, the conventional control, and four commercial reference varieties. All plants were grown in pots in a growth chamber established at 21 °C day/18 °C night with a 16 h photoperiod. The plants were arranged in a randomized complete block design with five replicates with 1 plant of each entry (MON 88302, conventional control and commercial reference varieties) per replicate. Once the plants started flowering a total of three newly opened flowers were collected from each plant. Pollen was extracted, combined among flowers collected from the same plot, and stained with Alexander's stain (Alexander, 1980). Pollen viability was evaluated for each sample, and pollen grain diameter was measured for ten representative viable pollen grains per replicate. General morphology of the pollen was observed for each of the five replicates of MON 88302, the conventional control, and the four commercial reference varieties (see Appendix H).

No statistically significant differences were detected ($\alpha = 0.05$) between MON 88302 and the conventional control for percent viable pollen or pollen grain diameter (Table VII-7). Furthermore, no visual differences in general pollen morphology were observed between MON 88302 and the conventional control. These results demonstrate that the introduction of the glyphosate-tolerance trait did not alter the overall morphology or pollen viability of MON 88302 compared to the conventional control. The pollen characterization data contribute to the detailed phenotypic description of MON 88302 compared to the conventional control. The result supports an overall conclusion that MON 88302 is comparable to conventional canola and is no more likely to pose a plant pest risk than conventional canola.

Table VII-7. Pollen Characteristics of MON 88302 Compared to the Conventional Control

Pollen Characteristic	Mean (S.E.)[1]		Reference Range[2]	
	MON 88302	Control	Minimum	Maximum
Viability (%)	99.8 (0.2)	100.0 (0.0)	98.0	99.8
Diameter (μm)	24.8 (0.1)	25.1 (0.1)	24.6	25.8

Note: No statistically significant differences were detected between MON 88302 and the conventional control ($\alpha = 0.05$).
[1]Means based on n = 5. S.E. = Standard Error
[2]Reference ranges were determined from the minimum and maximum mean value from among the four commercial reference varieties.

VII.D. Conclusions for Phenotypic, Agronomic, and Environmental Interactions Evaluation

An extensive and robust set of information and data were used to assess whether the introduction of the glyphosate-tolerance trait altered the plant pest potential of MON 88302 compared to the conventional control. Phenotypic, agronomic, and environmental interaction characteristics of MON 88302 were evaluated and compared to those of the conventional control and considered within the variation among commercial reference varieties. These assessments included five general data categories: 1) germination, dormancy, and emergence; 2) vegetative growth; 3) reproductive growth (including pollen characteristics); 4) seed retention on the plant and lodging; and 5) plant response to abiotic stress and interactions with diseases and arthropods. Results from the phenotypic, agronomic, and environmental interaction assessment demonstrate that MON 88302 does not possess: a) increased weediness characteristics; b) increased susceptibility or tolerance to specific abiotic stress, diseases, or arthropods; or c) characteristics that would confer a plant pest risk or a significant environmental impact compared to the conventional control.

VIII. U.S. AGRONOMIC PRACTICES

VIII.A. Introduction

As part of the plant pest assessment required by 7 CFR § 340.6(c)(4), impacts to agricultural and cultivation practices must be considered. This section provides a summary of current agronomic practices in the U.S. and North America for producing canola, and is included in this petition as a baseline to assess possible impacts to agricultural practices due to the cultivation of MON 88302. Discussions include canola production, canola seed production, plant growth and development, general management practices during the season, management of weeds, insects and diseases, canola rotational crops, and volunteer canola management. Information presented in Section VII.C.2 demonstrated that MON 88302 is no more susceptible to diseases or pests than conventional canola. Additionally data presented in Section VII.C show that, with the exception of tolerance to the herbicide glyphosate, MON 88302 is phenotypically equivalent to conventional canola. Thus, there are no changes to the inputs needed for MON 88302, and no impacts to most of the agronomic practices employed for production of canola. Discussion will also be provided on the current use of herbicide-tolerant canola and the expected use of MON 88302. In the areas where there is potential for impact on agronomic practices from the deregulation of MON 88302, the scope and magnitude of those impacts will be discussed.

Almost all the canola production in the U.S. is reported from eight states with the majority (88%) being produced in North Dakota. Spring and winter canola varieties have been developed which permit production in the northern Great Plains, the southern Great Plains and Pacific Northwest regions of the U.S. The soil types, moisture and temperature requirements for producing canola are generally similar to those for wheat. Spring canola is generally produced in areas with dry weather and shorter growing seasons while winter varieties can be produced further south to take advantage of longer growing seasons. Proper seedbed preparation, good genetics, proper planting dates and density and good integrated pest management practices are important to optimizing the yield potential and economic returns of canola.

Annual and perennial weed infestations are a serious obstacle to maximum yield potential in canola. Weeds compete with canola for water, nutrients and light resulting in substantial yield losses when left uncontrolled. Weed species in canola vary from region to region. Tillage, crop rotations, cultural practices and herbicides are all employed for weed management in canola. Early season weed control, particularly for spring canola varieties, is most important to minimize weed competition and yield losses in canola. Several selective herbicides are available for pre-emergence and post-emergence control of annual and perennial weeds in canola. Herbicide-tolerant canola varieties are utilized extensively which permit the in-crop post-emergence use of glyphosate, glufosinate and imazamox.

When canola volunteer plants are found in a field, they can easily be controlled using mechanical and chemical methods. Insect pests and diseases can also be considered

problematic in canola fields and can be managed using chemical means and crop rotation practices.

VIII.B. Overview of U.S. Canola Production

VIII.B.1. Canola Production

Brassica napus or oilseed rape is believed to have originated in the Mediterranean (Brown et al., 2008) and was cultivated by ancient civilizations in Asia and the Mediterranean and its oil was used for lighting (Colton and Sykes, 1992). It was reportedly grown in Europe for lamp oil and lubrication in the 13th century, and in Asia for cooking oil for thousands of years (Boyles et al., 2009). Demand for *B. napus* industrial grade oil increased during World War II when it became the oil of choice for the lubrication of steam engines. Diesel engines replaced steam engines at the end of World War II and demand plummeted. Researchers began investigating other market opportunities for oilseed rape. The high erucic acid content of the industrial oil was not desirable for human consumption. In addition, the meal derived from oilseed rape had relatively high concentrations of glucosinolates which reduced the animal feed value of the meal. In the 1960s, through intensive breeding programs, Canadian scientists made two important genetic modifications to oilseed rape which lead to the first double-low (low-erucic acid and low glucosinolate) variety (Brown et al., 2008). In 1978, to distinguish this new edible variety of *B. napus* oil from industrial *B. napus* oil, the Canola Council of Canada (formerly known as the Rapeseed Association of Canada) chose the word "canola" (Canadian oil, low acid) to become the registered trademark for edible *B. napus* oil (Brown et al., 2008) with less than 2% erucic acid in the oil (Codex Alimentarius, 2005). In 1985, the U.S. Food and Drug Administration (FDA) granted Generally Recognized As Safe (GRAS) status to canola oil (U.S. FDA, 1988) with these same characteristics which greatly increased the edible canola oil market in the U.S. In addition, canola varieties contain less than 30 µmol of aliphatic glucosinolates per gram of oil-free seed meal, making canola meal more attractive as an animal feed (Brown et al., 2008).

There are three species of *Brassica* that produce canola-quality oil: *Brassica napus* L. (oilseed rape), *Brassica rapa* (field mustard; also known as *Brassica campestris* L.) and *Brassica juncea* L. (mustard greens) (Codex Alimentarius, 2005). Both spring and winter canola varieties are available in *B. napus* and *B. rapa* while *B. juncea* has spring canola varieties only. Spring canola is a cool season crop that is grown in the northern states while winter canola varieties that are planted in the fall and require vernalization (winter chilling) to flower, are planted more in the Great Plains, Pacific Northwest, and Midwest regions of the U.S. *Brassica napus* is currently grown as an oilseed crop in Canada, China, Europe, India, Pakistan, Australia, and in the U.S. (Brown et al., 2008).

Canola oil is high quality oil that is used in a variety of foods including frying and baking oils, salad oils, margarines and shortenings and is the most valuable component of canola seed. It is the world's third largest source of vegetable oil with 15% of world vegetable oil consumption after soybean oil at 28% and palm oil at 32% (ASA, 2010; USDA-ERS, 2010). Canola oil appeals to health conscious consumers because it

contains a low level (7%) of saturated fatty acids which have been shown to increase blood cholesterol levels; a high level (approximately 60%) of the monounsaturated fatty acid, oleic acid, which has been shown to reduce serum cholesterol levels; a moderate level (approximately 20%) of linoleic acid, and an appreciable amount (approximately 10%) of alpha-linolenic acid relative to other oils (CCC, 2010) that are essential to human health and must be supplied in the diet. Canola oil has well established heart health benefits. The FDA has confirmed GRAS status for canola oil (U.S. FDA, 1988), and has issued a qualified health claim based on its ability to reduce the risk of coronary heart disease (U.S. FDA, 2006). Approximately 70% of Canada's canola oil is exported to the U.S. (CRB, 2008). Canola seed is also processed into canola meal which is used as high protein animal feed and is the second largest protein meal source produced in the world (CCC, 2009). However, it is relatively small compared to soybean meal. Global production of canola meal was 30.8 million metric tons in 2008/2009 compared to 151.6 million metric tons for soybean meal (USDA-ERS, 2010).

The European Union, Canada, and China are the largest producers of oilseed rape with 19.0, 12.64, and 12.10 million metric tons, respectively. The U.S. produced only about 0.66 million metric tons (1.1%) of the world's canola production (57.88 million metric tons) in the growing season 2008/2009 (USDA-FAS, 2010).

U.S. canola acreage in the past ten years has varied from approximately 827,000 to 1,524,000 acres with the lowest acreage recorded in 2009 and the highest in 2010 (Table VIII-1). Average canola yields for the U.S. have varied from 1,197 to 1,811 pounds per acre. Canola production ranged from 1.34 to 2.45 billion pounds over the past ten years with 2010 being the largest production year on record (Table VIII-1). According to data from USDA-NASS (USDA-NASS, 2011a), canola was planted on approximately 1.5M acres in the U.S. in 2010 producing 2.45 billion pounds of canola (Table VIII-2). The average yield in 2010 of 1,713 pounds per acre was the second highest yield per acre ever reported. The value of canola reached $486.9 million in the U.S. in 2010. In comparison, maize, soybean and wheat values in 2010 were $66.65, 38.92, and $12.99 billion, respectively (USDA-NASS, 2011b).

Canola can be grown on a wide range of soil types, but well-drained, clay-loam soils that do not crust are ideal for canola (NDSU, 2005). Canola cannot tolerate standing water or water-logged soils, and has moisture requirements similar to small grains, but is less tolerant to drought conditions. Only 13,535 (1.1%) of canola acres were irrigated in 2007 (USDA-ERS, 2010). Due to these factors canola fits well into rotations with small grain cereal crops (winter and spring wheat and spring barley).

U.S. canola is grown in three geographical regions – the Northern and Southern Great Plains and the Pacific Northwest (Table VIII-2). Approximately 730,000 acres or 88.3% of the U.S. canola production was grown in North Dakota in 2009 where a dryer, shorter growing season makes maize and soybean production less attractive (Figure VIII-1). Nearly all the canola grown in North Dakota is spring-sown *B. napus* (NDSU, 2005). Oklahoma had the second largest acreage with 42,000 acres which is planted primarily with winter canola varieties.

In the remaining acres reported, Minnesota and Montana tend to plant spring canola varieties, while Oregon, Colorado, Kansas, and Washington usually plant winter canola varieties. In past years, limited acres of canola were grown in many of the Midwest states and Southeastern states. However, the profit potential of maize and soybean has been more favorable than canola in these areas in recent years and canola acreage has declined accordingly. Oregon produced the highest average yield of 2,550 pounds per acre in 2009 with North Dakota coming in second with 1,840 pounds per acre. The lowest average yield was recorded in Oklahoma with 1,300 pounds per acre.

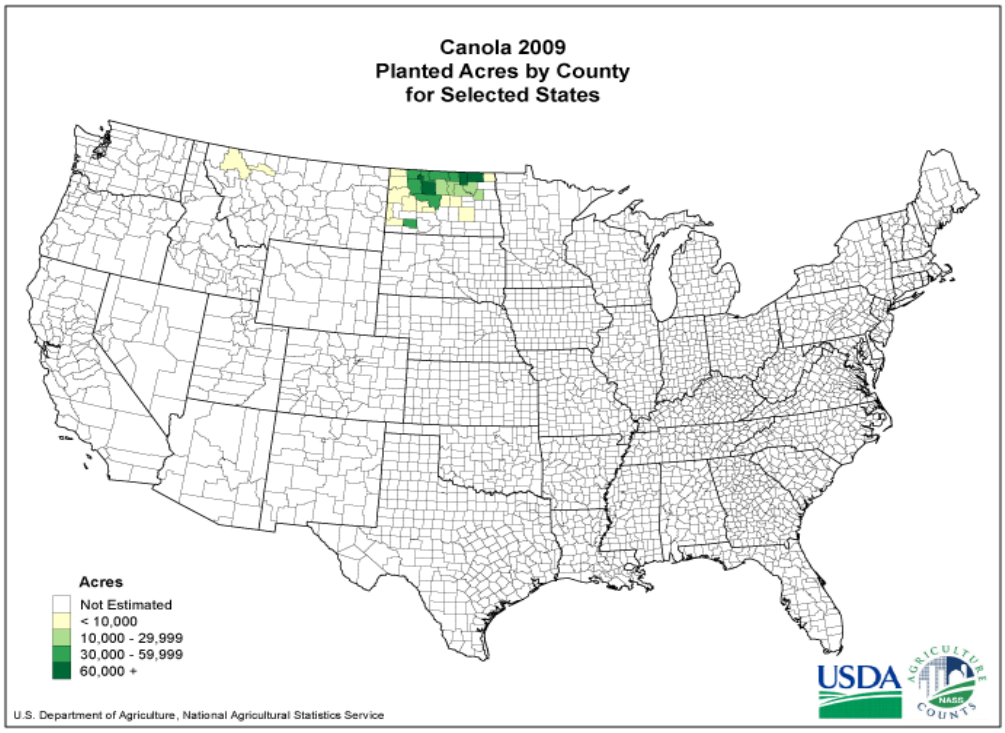


Figure VIII-1. Planted Canola Acres by County in the U.S. in 2009
Source: http://www.nass.usda.gov/Charts_and_Maps/Crops_County/cn-pl.asp.

Table VIII-1. Canola Production in the United States, 2001 – 2010[1]

Year	Acres Planted	Acres Harvested	Yield (lbs/acre)	Production (thousand pounds)	Value (millions of dollars)
2010	1,524,000	1,431,000	1,713	2,450,947	486.9
2009	827,000	814,000	1,811	1,474,130	238.9
2008	1,011,000	989,000	1,461	1,445,064	271.0
2007	1,176,000	1,156,000	1,238	1,430,734	260.3
2006	1,044,000	1,021,000	1,366	1,394,312	165.5
2005	1,159,000	1,114,000	1,419	1,580,985	152.0
2004	865,000	828,000	1,618	1,336,530	143.9
2003	1,082,000	1,068,000	1,416	1,512,250	159.9
2002	1,460,000	1,281,000	1,197	1,533,420	162.7
2001	1,494,000	1,455,000	1,374	1,998,515	175.4

[1]Source: (USDA-NASS, 2011c)

Table VIII-2. U.S. Canola Production by State in 2010

Region/State	Acres Planted[1]	Acres Harvested[1]	Average Yield[1] (pounds/acre)	Total Production[1] (thousands pounds)	Value[2] (millions of dollars)
Idaho	19,500	18,400	1,800	33,120	6.2
Minnesota	46,000	45,000	1,530	68,850	(ND)
Montana	17,500	17,400	1,730	30,102	6.0
North Dakota	1,280,000	1,270,000	1,720	2,184,400	436.9
Oklahoma	60,000	56,000	1,600	89,600	15.5
Oregon	6,000	5,700	2,450	13,965	2.5
Other States[3]	19,800	18,500	1,671	30,910	19.8
U.S. Totals	**1,448,800**	**1,431,000**	**1,713**	**2,450,947**	**486.9**

(ND) Not disclosed
[1](USDA-NASS, 2011a)
[2](USDA-NASS, 2011b)
[3] Other states include Colorado, Kansas, and Washington.

Managing input costs is a major component to the economics of producing a canola crop. The key decisions on input costs include choosing what seed or canola varieties to plant, amounts of fertilizer to apply and what herbicide program to use. The average gross return for producing canola in North Dakota was $250.89 per acre for the years 2005-2009 according to statistics compiled by Farm Management Specialists (Table VIII-3). The total direct and overhead expenses were $179.52 and $27.44 per acre, respectively. The net return for the five-year period averaged $43.94 per acre. With an average government payment of $11.33 per acre the net return was $55.27 per acre.

Table VIII-3. North Dakota Canola Production Costs and Returns from 2005 – 2009[1]

Yield:1546 lbs./Acre	
Market Value: $15.27 per cwt	
Product Income	237.62
Crop Insurance & Other Income	13.27
Total Income	**250.89**
Direct Expenses:	
Seed	31.06
Fertilizer	43.56
Crop Chemicals	22.69
Crop Insurance	13.14
Fuel and Oil	13.19
Repairs	13.60
Custom Hire	3.74
Land Rent	34.50
Operating Interest	3.67
Miscellaneous	0.36
Total Direct Expenses	**179.52**
Return Over Direct Expenses	**71.38**
Overhead Expenses:	
Hired Labor	3.61
Machinery Leases	2.35
Farm Insurance	2.26
Utilities	1.63
Dues & Professional Fees	0.53
Interest	3.12
Machinery & Building Depreciation	11.86
Miscellaneous	2.09
Total Overhead Expenses	**27.44**
Total & Direct Overhead Expenses	**206.95**
Net Return	**43.94**
Net Return with Government Payment ($11.33)	**55.27**

[1](FINBIN, 2010). Supporting Information: Cash rent farms only, total number of fields: 540, total number of farms: 315, and average size of fields: 166 acres

VIII.B.2. Canola Seed Production

Standardized seed production practices are responsible for maintaining high-quality seed stocks, an essential basis for U.S. agriculture. By the early 20th century, agronomists learned how to develop specific plant varieties with desirable traits. In the U.S., state agricultural experiment stations developed many seed varieties that were distributed to growers for use. Seed was saved by growers and later sold to neighbors; however, the desirable traits of the varieties often were lost through random genetic changes and contamination with other crop and weed seed (Sundstrom et al., 2002). The value of seed quality (including genetic purity, vigor, weed seed presence, seed-borne diseases, and inert materials, such as dirt) was quickly identified as a major factor in crop yields. States in the U.S. developed seed laws and certification agencies to ensure that purchasers who received certified seed could be assured that the seed met established seed quality standards (Bradford, 2006). The federal government passed the U.S. Federal Seed Act of 1939 to recognize seed certification and official certifying agencies. Regulations first adopted in 1969 under the Federal Seed Act recognize land history, field isolation, and varietal purity standards for foundation, registered, and certified seed. Under international agreements such as the Organization for Economic Co-Operation and Development (OECD) scheme, the U.S. and other countries mutually recognize minimum seed quality standards (Bradford, 2006). The Association of Official Seed Certifying Agencies (AOSCA) represents state and private seed certification in the U.S., and includes international member countries in North and South America, Australia, and New Zealand.

Canola seed is broadly separated into three seed classes: 1) breeder; 2) foundation; and 3) certified (CCC, 2011). Breeder seed is seed directly controlled by the originating or sponsoring plant breeding organization or firm responsible for the maintenance of that variety. Foundation seed is first-generation seed increased from breeder seed and is handled to maintain specific varietal purity and identity. Certified seed is the progeny of breeder or foundation seed, and is the class recommended for commercial canola production. Not all canola seed sold may be officially certified; however, commercial canola seed sold and planted for normal canola production is produced predominately to meet or exceed certified seed standards. This section of the petition will provide a broad overview of the standards (AOSCA, 2009) and practices used in producing certified canola seed.

Seed breeders and producers have put in place practical measures to assure the quality and genetic purity of seed varieties for commercial planting. The need for such systems arose from the recognition that the quality of improved seed varieties quickly deteriorated in the absence of monitoring for quality and genetic purity (CAST, 2007). Seed certification programs were initiated in the early 1900s in the U.S. to preserve the genetic identity and varietal purity of seed. There are special land requirements, seed stock eligibility requirements, field inspections and seed labeling standards for seed certification. Seed certification services are available through various state agencies affiliated with the AOSCA. Large seed producers implement their own seed quality assurance programs. However, large seed producers often will utilize the services of

state certifying agencies as a third party source to perform certain field inspections and audits.

The U.S. canola production for all purposes has varied from approximately 0.8 to 1.5 million acres in the past ten years (USDA-NASS, 2011c) Table VIII-1). Based on an average seeding rate of five pounds per acre, approximately 7.5 million pounds of canola seed would be required to plant 1.5 million acres. Additional seed volume would be required to make allowances for seed losses due to weather, poor yields, and quality issues. Additional allowances would be required for distribution excess, seed returns, replants, and potential increases in canola acreage. Approximately 5,000 acres of commercial seed production would supply sufficient seed to plant the entire U.S. canola acreage with the various production and distribution allowances mentioned above (Bateman, Monsanto - Personal Communication, 3/17/11).

Canola seed production involves a three stage process. The first stage or pre-foundation seed stage occurs in isolated tents for complete pollination control. Individual inbred plants are grown and tested to ensure the plants meet the desired genetic characteristics and profile. The next stage or foundation seed stage occurs in highly isolated open fields. During this stage inbred seed lines are further tested to ensure the desired genetic characteristics and purity are maintained under field conditions. The final stage involves the production of hybrid seed or open-pollinated seed for commercial seed sales. Most of the hybrid and open-pollinated seed is produced in Southern Alberta, Canada and Northwestern U.S. These are preferred locations for seed production because of ideal heat units, accessibility to irrigation, and distance from commercial canola fields (Monsanto Company, 2009). To meet the demand for hybrid seed and to minimize production risks, most seed companies have off-season seed production locations in the Southwestern U.S. and Chile.

Canola is a relatively easy crop to manipulate genetically and many new varieties with new quality and agronomic characteristics are available from universities and private seed companies (NDSU, 2005). Canola seed varieties are developed from three different breeding techniques, namely open-pollinated, synthetic hybrids, and hybrids (NDSU, 2005). Over 95% of the acreage in the U.S. is planted with hybrid canola varieties (Bonnetta, Monsanto Company – Personal Communication, 8/22/2010). Hybrid canola varieties generally have higher yield potentials, but also have higher seed production costs. Hybrid canola varieties are produced from a cross between two genetically different inbred parent lines providing hybrid vigor (heterosis).

Producing hybrid canola requires using a pollination control system to prevent unwanted self-pollination. Three primary pollination control methods used in North America: 1) the biotechnology derived male sterility system; 2) the NPZ MSL (Lembke) system ; and 3) the INRA-ogura cytoplasmic male sterility (CMS) system (Stiewe et al., 2010). The biotechnology-derived system uses tapetal cell-specific male sterility and fertility restorer genes to facilitate hybrid seed production (Mariani et al., 1992). The MSL-system (Male Sterility Lembke) is a private system owned by NPZ/Lembke based on a spontaneous mutant selected in the NPZ nursery (Frauen and Paulmann, 1999). The INRA-ogura system uses a CMS and restorer gene transferred from *Raphanus* (Buzza, 1995). The

CMS system is composed of three lines, usually referred to as the A line (male sterile or female parent line), B line (maintainer line) and R line (restorer or male parent line). The A and B lines have similar genotypes, with the exception of the CMS gene, that confers male sterility to the A line. The B line has a cytoplasm which allows normal pollen production facilitating the breeding process (Buzza, 1995). The first step in the hybrid seed production process involves foundation seed crossing block where female parent Line A is pollinated by the genetically identical Line B to produce sufficient quantities of Line A seed. The second step involves pollination of Line A with restorer Line R to produce fully fertile F1 hybrid seed (Thomas, 2003b).

Special planting methods are utilized in the production of hybrid canola seed involving planting alternating strips of male and female lines. The ratio for male to female varies with seed producer and variety. The male rows are destroyed after flowering and only the female blocks are harvested for seed.

Canola seed is produced by several companies that produce and sell seed, such as Monsanto Company, Pioneer Hi-Bred International, Bayer CropScience, Croplan Genetics, and Dow AgroSciences. Seed companies in turn contract acreage with growers to produce the required amount of canola seed. Seed companies identify top canola growers to produce the seed and also monitor and inspect seed fields throughout the growing season. Seed companies have processing facilities and/or a network of tollers to clean, condition, and bag the harvested canola seed as well as monitor and inspect all the processes at the plant.

The entire seed production process for the majority of the seed companies and tollers is certified by the International Organization for Standardization (ISO) and includes internal and external audits (ISO, 2009). ISO standards ensure desirable characteristics of seeds and services, such as quality, safety, reliability, and efficiency. The ISO standards represent an international consensus on good management practices with the aim of ensuring that the organization can consistently deliver excellent product and services. The standards must meet the customer's requirements and applicable seed regulatory requirements, and must aim to enhance customer satisfaction.

The field operations and management practices for producing canola seed are similar to normal canola production. However, special attention is needed in certain areas to produce seed with high quality, high germination rates, and high genetic purity. General guidelines specific for seed production are discussed below.

A seed production field should not have been planted with canola the preceding five years in order to avoid volunteer canola plants and to ensure genetic purity. All seed stock should be treated with fungicides and insecticides to protect seedlings from various seedling diseases and flea beetle. Very early planting into cold soil conditions should be avoided because this can result in poor emergence and uneven stands. Every effort should be made to eliminate weeds in a seed field through the use of herbicides, cultivation, and hand weeding to prevent weed seed in the harvested canola seed. Roguing should be performed on all seed production fields to remove volunteer canola and off-type plants prior to or during flowering. Fields should be scouted frequently for

insect pests and insecticides should be applied when insect pest infestations reach economic threshold levels. Foliar-applied fungicides should be considered when disease infestations are predicted in the area. Honey bee hives should be placed in the seed fields to encourage pollination of the female parent line with pollen of the male parent line. Swathing (cutting the crop and placing it in rows directly on the cut stubble to facilitate drying rate, ensure even ripening and reduce the possibility of seed losses from wind and hail) and combining at harvesting should occur at optimum times to avoid seed shattering and damage to the seed. With large acreage of canola, swathing should start when seed color change is approximately 20% to 25% and the majority of the crop should be cut at or near the optimum seed color. Swathing should begin as early as 15% seed color change (NDSU, 2005). Combining should begin when the seed moisture drops below 10% and no green pods are visible (Boyles et al., 2009). Harvesting equipment should be adjusted to minimize or avoid seed damage and should be cleaned before entering the seed fields to minimize genetic contamination. Certain handling equipment, such as auger elevators, should be avoided because they can increase seed damage.

Field inspections are vital to ensure canola seed meets seed certification requirements, ISO certification standards, regulatory standards, and trait licensing agreement standards. Field inspections are conducted on seed production fields throughout the growing season to evaluate variety purity, ensure canola plants are developing properly, and fields are maintained free of weeds, insects, and diseases.

Production plant personnel make every effort to avoid mechanical damage to the harvested seed during the screening, cleaning, and bagging process. Specific methods are used to assure the genetic purity and identity of the seed is maintained throughout the handling and storage operation. Bin inspections and sample collections are conducted at storage locations at the plant to examine the physical characteristics of the canola seed and to ensure proper bin cleanout. Seed is inspected for appearance, disease, discoloration, seed coat, mechanical damage, inert matter, and weed seed. Germination, hybridity, and impurity are tested and quantified on all seed lots to verify acceptable levels and meet labeling requirements.

Commercially certified canola seed must meet state and federal seed standards and labeling requirements. AOSCA standards for certified canola seed require all seed fields be inspected in the early flowering stage to ensure the fields are isolated from other canola crop fields by at least 2640 feet (AOSCA, 2009). Fields must be free of prohibited noxious weeds. Inspections should also ensure that the number of off-type plants and plants of other varieties do not exceed 1.5 plants per 10,000 canola plants. Plants of other *Brassica* crop species must not exceed 1 plant per 10,000 canola plants. The percentage of hybrid seed shall not be less than 75%. State seed certification standards vary slightly from state to state and can be more restrictive than the seed standards of AOSCA.

When deregulated, MON 88302 seed will be produced in the same manner as commercially certified canola seed, such that it will meet all state and federal seed standards and labeling requirements.

VIII.C. Production Management Considerations

Pre-Season

There are generally three types of canola crops that can be grown in the U.S. and the grower must decide which of these types is best suited for his area and cropping system (Brown et al., 2008). Winter canola is planted in the fall, overwinters, requires vernalization (winter-chilling) to produce flowers, and is harvested the following summer. Winter canola is generally produced in the Pacific Northwest, southern Great Plains and Midwest regions of the U.S. There is a second type of winter canola that is planted in the fall and overwinters, but does not require vernalization to produce flowers. This winter type is produced in the southeast region of the U.S. The third type is spring canola which is planted in the early spring, requires no vernalization to flower, and is harvested in late summer. Spring canola is grown primarily in the northern Great Plains states including North Dakota, South Dakota, Minnesota, Montana, Idaho, and also in Washington. Winter and spring canola may require different agronomic practices and can be affected by different insect pests and diseases.

Crop rotation, tillage system, seed or variety selection(s), and soil fertility are management issues which require production decisions well in advance of planting the canola crop. Many of the decisions in this area are made immediately after harvest of the previous crop or sooner, particularly with winter canola production. There are many benefits to crop rotation and the majority of the canola acreage is planted in rotations with small grain cereal crops. Crop rotation is generally a long term decision, but the rotation sequence can be modified to take advantage of a particular economic or market opportunity. Canola is commonly grown following small grain cereal crops (winter or spring wheat, barley, maize or sorghum) (Brown et al., 2008). Growers are advised not to plant canola following canola to avoid a build-up of soil-borne diseases like Sclerotinia stem rot, blackleg, or club root. Ideally, canola should be planted only once every four years to minimize diseases. Also, growers are advised to avoid planting canola after legume crops or sunflower which is susceptible to Sclerotinia stem rot, or Rhizoctonia and Fusarium root rots.

Canola is highly sensitive to certain herbicide residues in the soil, particularly sulfonylurea herbicide residues (Boyles et al., 2009). Therefore only sulfonylurea-tolerant varieties should be planted where sulfonylurea herbicides were used in the previous year. Additionally, growers must know the herbicide use history and plant back restrictions before planting canola in any field to avoid severe crop injury and/or yield loss due to carryover herbicide residues. For example, bromoxynil has only a 30-day plant back restriction for canola, but imazethapyr has a restriction of 40 months for most varieties. In addition to the plant back restriction, some herbicide labels require a field bioassay to determine whether canola can be planted without risk of crop injury. Imazethapr herbicide is used often in legume crops in the Pacific Northwest and crop injury to canola has been reported six years after an imazethapyr application (Brown et al., 2008).

Canola can be grown with conventional tillage, conservation tillage, or direct-seeded into small grain straw stubble in a no-tillage cropping system (Brown et al., 2008). The benefits of conservation tillage or no-till systems include reduced soil erosion, reduced fuel and labor costs, soil moisture conservation, and improved soil structure and organic matter content (Brown et al., 2008).

Primary and secondary pre-plant tillage helps remove crop residue, control weeds and volunteer plants from the previous crop and is an important consideration prior to adopting no-till or direct-seeding systems for canola. Regardless of the tillage system utilized by the grower, the tillage system must facilitate good stand establishment and emergence. Good seed-to-soil contact is essential for good seed germination and uniform emergence that are critical to obtaining maximum yields (Brown et al., 2008). Good, uniform stands of canola will also be an important component of weed management during the growing season. Conservation and no-tillage systems leave crop residue such as small grain straw from the previous crop which is desirable for erosion control and moisture conservation. However, no-till planting or direct seeding into heavy crop residue can make it difficult to achieve good seed-to-soil contact and result in poor emergence of canola. These factors must be weighed against the fact that additional tillage will generally result in additional soil moisture loss, and an increased probability of soil crusting before seedling emergence.

Spring canola has provided good performance under conservation tillage while winter canola has generally performed poorly with conservation tillage systems (Brown et al., 2008). Winter canola is not recommended when planting into excessive amounts of fresh straw, or when soil temperatures are lower than average in the fall months, because it can result in poor emergence. Seed yields with direct-seeded winter canola have been significantly lower than with conventional tillage systems (Brown et al., 2008). Spring canola is better suited to conservation tillage due to better soil moisture conservation and availability. Spring canola has fewer problems dealing with heavy straw residue and, residue from the previous crop has usually decomposed to some degree during the winter months prior to planting spring canola. In addition, cooler soil temperature can be advantageous to spring canola. The Conservation Tillage Information Center (CTIC, 2008) reports that conservation tillage (no-till or mulch-till) is used on approximately 32% of the canola acres in North Dakota. Crop Specialists at North Dakota State University indicate that the amount of direct-seeded or no-till canola in North Dakota varies across the state based on rainfall. In the eastern or Red River Valley area where rainfall is relatively high, only about 5% is direct-seeded into wheat straw (Kandel – NDSU, Personal Communication, 7/23/2010). The drier, central area and even drier, western area of the state are estimated to have approximately 80% and 100% of the acreage direct-seeded, respectively.

Effective nutrient management and maintainance of good soil fertility is essential for high yielding and high quality canola. Nitrogen, phosphorus, potassium, sulfur, and boron, depending on the region, are the most limiting nutrients for successful canola production (Brown et al., 2008). Soil sampling and testing is the first step in assessing soil nutrient levels and the requirements for supplemental fertilizers. The availability of soil nutrients is dependent on soil acidity or the pH level and must be included in this assessment. The

ideal soil pH for growing canola is between 6.0 and 7.0. Canola yields will be adversely affected when the soil pH is below 5.5 (Brown et al., 2008).

Nitrogen is the most limiting of all plant nutrients in canola and sufficient nitrogen must be available to the plant at every growth stage. Supplemental nitrogen requirements for North Dakota are based on the yield potential of canola, nitrate nitrogen available in the soil at 0-24 inch depth and the previous crop credit for nitrogen (Franzen and Lukach, 2007).

Research studies have shown that nitrogen recommendations can be capped at 150 lbs. per acre in the cooler, moister areas of the state without impacting yield. In the drier, warmer areas of the state nitrogen rates can be capped at 120 lbs per acre. Canola is very sensitive to fertilizer salts and no more than 5 lbs. of nitrogen per acre is recommended for placement with the seed at planting on medium-textured soils (Franzen and Lukach, 2007).

Canola has a moderate requirement for phosphorus and phosphorus fertilizer rates are based on soil tests. Phosphorus is not mobile in the soil and should be banded with the seed at planting or incorporated into the soil before planting (Brown et al., 2008). A starter fertilizer rate of 20-30 lbs. of P_2O_5 per acre is generally sufficient for most soil test levels unless the grower intends to build up phosphorus levels in the soil (Franzen and Lukach, 2007). Potassium requirements are also based on soil tests. Many soils contain sufficient levels of potassium requiring no potassium fertilizers. Potassium applications are not needed when the soil test indicates 160 ppm or more of potassium (Franzen and Lukach, 2007). Potassium can also be applied as a starter fertilizer with or alongside the seed at planting.

Canola has special requirements for sulfur and it is often the second most limiting nutrient in canola production (Brown et al., 2008). Sulfur deficiencies result in yellowing between leaf veins, cupped leaves and stunting (Franzen and Lukach, 2007). In addition, in the presence of sulfur deficiency flowering is delayed, seed often does not set, and pods will be barren or poorly developed. North Dakota studies have demonstrated significant yield increases from sulfur applications (Franzen and Lukach, 2007). Since soil tests tend to overestimate available sulfate and are highly variable, North Dakota specialists recommend 20 to 30 pounds of sulfur per acre when medium to low levels of sulfur are detected and 10 to 15 lbs of sulfur per acre when high levels of sulfur are detected (Franzen and Lukach, 2007). The sulfur fertilizer should be in the form of ammonium sulfate, ammonium thiosulfate or potassium thiosulfate since canola takes up sulfate sulfur.

Canola requires more boron than most other crops. Boron at 1 to 2 pounds per acre should be broadcast when the soil tests show less than 0.5 ppm boron (Brown et al., 2008). Canola has not shown yield responses to applications of micronutrients such as chlorine, copper, iron, manganese, molybdenum or zinc (Brown et al., 2008).

Many varieties are available for each canola type, and variety selection is one of the most important decisions a grower makes in growing a successful crop (NDSU, 2005). The

performance of a given variety may differ from year to year and location to location due to changing environmental conditions. Therefore, growers rely on variety performance trials conducted by universities and private companies across a number of locations and years to make a selection. There are several factors to consider in choosing canola varieties, namely: yield, maturity, plant height and lodging, disease tolerance, and seedling vigor (NDSU, 2005).

Planting and Early Development

An understanding of the growth stages of canola is important for the proper timing of certain management practices such as herbicide and insecticide applications. In addition, the impact of certain weather conditions and diseases on canola yield is dependent on growth stage. Temperature, moisture, light, nutrition and variety influence the length of each growth stage (NDSU, 2005). The growth stage key utilized by BBCH (2001) outlines nine main growth stages: Stage 0 - Germination, Stage 1 – Leaf Development, Stage 2 – Formation of Side Shoots, Stage 3 –Stem Elongation, Stage 5 – Inflorescence Emergence, Stage 6 – Flowering, Stage 7 – Development of the Fruit, Stage 8 – Ripening and Stage 9 - Senescence.

The pre-emergence stage or germination process typically takes from four to ten days depending on soil temperature and moisture, seed-soil contact and depth of planting (NDSU, 2005). Canola is susceptible to many soil-borne pathogens during this stage. Planting high quality seed is essential for controlling diseases. University specialists recommend growers always plant certified canola seed that is free of diseases to prevent the spread of seed-borne blackleg (*Leptosphaeria maculans*), Sclerotinia stem rot (*Sclerotinia sclerotinium*), and Alternaria black spot (*Alternaria spp*.) (Brown et al., 2008). Fungicide seed treatments are economically beneficial for protecting seedlings from seed rot, damping off, seedling blight, and early season root rot caused by *Pythium, Rhizoctonia*, and *Fusarium*. The fungicide seed treatments are also effective protection against seed-borne blackleg and Alternaria black spot.

During the seedling stage, canola plants are also very susceptible to flea beetle species (*Phyllotreta cruciferae* and *P. strilolata*) infestations. Insecticide seed treatments provide protection from flea beetle species feeding for most of the susceptible seedling stage (NDSU, 2005). Some of the seed treatments registered for canola contain multiple fungicides for broad spectrum disease protection plus an insecticide for control of flea beetles. For example, Prosper 400 contains the fungicides thiram, carboxin, and metalaxyl plus the insecticide clothianidin. Helix XTra and Helix Lite contain the fungicides difenoconazole, mefenoxam, and fludioxinil plus the insecticide thiamethoxam (Brown et al., 2008).

The exposed growing point of spring canola makes the seedlings more susceptible to spring frosts, soil drifting, and hail damage. Freezing temperatures in the spring can cause serious injury to canola plants. The amount of frost injury will depend on soil moisture conditions, the rate at which thawing occurs, the growth stage of the plants and the amount of cold temperature-hardening that the plant is exposed to prior to freezing temperatures (NDSU, 2005). Spring canola that is exposed to several days of near

freezing temperatures will undergo a gradual hardening process that will allow the plants to withstand freezing temperatures without serious damage.

The minimum soil temperature for canola to germinate is 38 °F (NDSU, 2005). The length of time needed for emergence is dependent on soil temperature. Canola will emerge in 17 to 21 days from planting when the average soil temperature is in the low 40s. When the soil temperature averages in the low 50s, canola will emerge in approximately 10 days. In the early years of practicing conservation tillage, if was found that heavy straw residues from the previous crop can result in lower soil temperature and higher soil moisture which could delay planting (Brown et al., 2008). However, water infiltration improves after utilizing no-tillage or direct-seeded systems for several years. Frequently, that allows growers to enter fields sooner, plant earlier and establish the crop earlier. For optimum seed germination, seeding should be delayed until soil temperatures exceed 49 °F (Brown et al., 2008). Seeding into cooler soil temperatures will cause slow and uneven germination, thin or uneven stands and fosters weed competition.

Seeding date of spring canola will have an effect on time of germination, crop emergence and establishment, days from planting to flowering, plant height, maturity, and final yield (Brown et al., 2008). Spring canola should be planted in April to early May in North Dakota to maximize yields (NDSU, 2005). Spring canola should be planted prior to planting spring cereal grains. Research studies have shown significant reductions in yield when planting is delayed beyond May 1 in southwestern North Dakota and May 15 in other regions of the state (NDSU, 2005). Delaying planting much beyond these dates will increase the chances of encountering high temperatures and/or drought conditions during flowering when canola is susceptible to heat and drought stress.

Since winter canola is planted in the fall, planting dates should be selected to provide time for plants to develop sufficient growth before the onset of winter to minimize winter damage (Brown et al., 2008). Generally, winter canola should be planted six weeks before the first killing frost in most states (Boyles et al., 2009; Brown et al., 2008). The goal is to have at least 45 days of growth or to have plants with four to six fully opened leaves (in a rosette stage of growth) before winter. Planting too early in the fall can result in crop failure as overly large plants deplete soil moisture. This moisture stress may cause the plants to flower in the fall which makes them more susceptible to winter kill. On the other hand, planting too late will result in seedlings that are too small that have insufficient reserves to survive winter conditions and/or compete well with weeds in the spring. Planting dates for optimum yields with winter canola in the Pacific Northwest are August 14 to 24 although planting can be in early August to the second week of September (Brown et al., 2008). Recommended planting dates for winter canola in the Great Plains vary according to latitude. For example, optimum planting dates for Kansas and Oklahoma are August 26 through September 25 and August 20 to September 21 respectively (Brown et al., 2008).

Canola is adapted to a wide range of plant population densities. The optimum seeding rate for spring canola is 600,000 pure live seeds (PLS) per acre which equates to 16 PLS per square foot (NDSU, 2005). This seeding rate should result in an optimum established stand of 8 to 14 plants per square foot. Similar seeding rates are recommending in winter

canola. The seeding rate for winter canola should result in 10 to 16 established seedlings per square foot (Brown et al., 2008). Significant yield differences do not occur unless populations at harvest are less than one or greater than 15 plants per square foot (Boyles et al., 2009). Low plant populations of canola will not compete as effectively with weeds, and can produce lower yields although canola plants in low planting densities have the ability to compensate (Brown et al., 2008). Individual plants in low plant densities will have broader stems and greater raceme branching because of less intra-crop competition. Plant populations that are too high will result in thin-stemmed plants, high intra-crop competition, crop lodging at maturity and decreased yields. Planting into poor soil and seedbed conditions will require increased seeding rates to compensate for poor seedling emergence. Direct-seeding or no-tilling spring canola into standing wheat straw requires a 10 to 15% increase in seeding rate to achieve the same plant stand compared to well-cultivated and firm seedbed conditions (Brown et al., 2008).

To determine pounds of canola seed to plant in order to achieve the optimum seeding rates presented above, it is important to know the number of seeds per pound for the specific variety of canola being planted. Canola seeds are small and round with approximately 75,000 to over 200,000 seeds per pound dependent on the type of canola and variety (NDSU, 2005). Generally, the hybrid varieties of *B. napus* canola will contain 75,000 to 100,000 seeds per pound. Open-pollinated *B. napus* canola varieties will vary from 135,000 to 160,000 seeds per pound. *Brassica rapa* canola varieties usually contain more than 200,000 seeds per pound. Certified canola seed bags will indicate the number of seeds per pound and seed germination percentage which is vital information in determining the actual seeding rate. Seeding rates will generally be 4 to 8 pounds per acre (NDSU, 2005).

Most commercial grain drills used to plant small grain cereals are acceptable for planting canola (Brown et al., 2008). The narrower row spacing provides quicker canopy closure, reduces weed competition, and lessens wind shattering before harvest (Boyles et al., 2009). Canola should be seeded to a depth that allows the seed to be covered and must be planted into moist soil. The best germination and emergence usually occurs with seeding depths of 0.5 to 1 inch (Boyles et al., 2009; NDSU, 2005). Canola has difficulty emerging through thick soil covers or crusted soil (Boyles et al., 2009).

Early season weed control is important to minimize yield losses in canola (Boyles et al., 2009). Canola in the seedling stage is a poor competitor with weeds and becomes more competitive as it approaches the late rosette and bolting stage (NDSU, 2005). Planting canola into a weed-free seedbed is essential and can be achieved with tillage, herbicides, or a combination of both methods. Seedbed conditions that provide rapid germination and early emergence are important for uniform stand establishment and minimize competition with weeds. Uniform stands of canola will be an effective weed control management tool as the growing season progresses. Effective weed management in canola relies heavily on selective post-emergent herbicides. The list of registered herbicides in the U.S. is relatively small compared to other major crops. Sethoxydim and clethodim are post-emergent in-crop herbicides for control of annual grasses while quizalofop will control annual grass and quackgrass (perennial). Clopyralid will control several broadleaf species in canola. Herbicide-tolerant canola varieties are also available

which allow the use of broadspectrum post-emergent herbicides such as glyphosate, glufosinate, and imazamox. Early canopy closure can reduce the number of in-crop herbicide applications.

Mid- to Late Season

The first true leaves of canola develop 4 to 8 days after emergence and plants quickly establish a rosette. The older leaves develop at the base increasing in size while smaller, younger leaves develop in the center. During the rosette stage rapid and abundant leaf growth occurs producing more dry matter per day and increasing yield potential. Bud formation is triggered as the days lengthen and temperatures rise. The plant reaches its maximum leaf area index in the late bud stage. The vegetative stages (seedling to first flower) for *B. napus* generally range from 40 to 60 days, depending on environmental conditions. Flowering begins with the opening of the lowest bud on the main stem and continues for the next 14 to 21 days. High temperatures together with moisture stress during this stage can severely reduce yield potential. Ripening begins when the petal on the last formed flower on the main stem falls. Seed fill is complete approximately 35 to 45 days after flower initiation.

Most insect pests of canola damage the flowers, leaves, developing buds, seed pods, or developing seed and cause yield reductions and often an associated reduction in oil content (Brown et al., 2008). Spring and winter canola is impacted by the same insect pests, but the degree of damage may be different. New generations of adult flea beetles can emerge in mid-July to early August (NDSU, 2005). Populations usually are not high enough to cause serious damage to canola. However, extremely high populations can feed on green pods causing pods to shatter and seeds to remain green and require treatment with insecticides. Cabbage seedpod weevil (*Ceutorhynchus assimilis*) is the major insect pest of winter canola (Brown et al., 2008). Other insect pests which occasionally will infest canola are diamondback moth (*Plutella xylostella*), bertha armyworm (*Mamestra configurata*), lygus bugs (*Lygus spp*.), cutworms, grasshoppers, blister beetles, and aphids (NDSU, 2005). Infestations may exceed economic threshold levels requiring insecticide treatments.

As previously mentioned, blackleg, Sclerotinia stem rot and Alternaria black spot are seed-borne diseases which can be effectively managed by planting certified seed with seed fungicide treatments. The first step in minimizing these diseases is to avoid growing canola within three years of another host crop species (Brown et al., 2008). Where blackleg infestations exist, planting blackleg-resistant varieties is an effective management practice. Foliar fungicide applications for control these diseases are not economical under most situations, although foliar applications of boscalid and azoxystrobin fungicides are effective on Sclerotinia stem rot and Alternaria black spot respectively as needed.

Harvest

Spring canola is potentially at risk for frost damage in the fall. It is important to have stands of canola that ripen early and uniformly since the amount of fall frost damage

depends on the stage of maturity (NDSU, 2005). A temperature of 27 °F is enough to kill immature seeds containing 50% to 60% moisture while seeds at 35% moisture will escape damage (NDSU, 2005). Frost fixes the chlorophyll or green color in immature seed and can increase the cost of refining the oil (Brown et al., 2008; NDSU, 2007). Canola seeds in all pods on a plant reach physiological maturity and complete filling at about 40% moisture. They then slowly turn from green to light yellow, or reddish-brown, brown or black (NDSU, 2005). With dry weather and temperatures of 90°F, canola seed can have a 10% to 50% seed color change in three to five days or less. Seeds lose moisture at the rate of about 2% to 3% or more each day depending on the weather. Spring varieties of canola usually mature 85 to 110 days after planting, depending on the variety and environmental conditions (NDSU, 2005). Pod shattering in standing canola due to excessive wind, rain, and hail can result in yield losses of greater than 50% when the crop is ripe and ready to harvest (Boyles et al., 2009). Therefore, if possible, canola should be harvested immediately upon ripening to prevent pre-harvest losses.

Most canola is harvested using one of two methods: 1) direct combining of standing canola, and 2) swathing and combining. In the latter method, the canola swath is allowed to cure and ripen for a minimum of 10 to 14 days before harvesting which hastens maturity and avoids frost damage in areas with a short growing season (Boyles et al., 2009). The direct combining method does not require additional equipment (swather) for growers. There has been considerable research and debate on which method is best for harvesting canola. Direct combining standing canola has resulted in less green seed and generally higher oil content and test weight than the swathing and combining method in multi-year research studies in North Dakota (NDSU, 2007). However, canola is more vulnerable to seed and shatter loss when direct combining is delayed past the optimum time. Swathing can be advantageous since it facilitates harvest 8 to 10 days earlier (Boyles et al., 2009). Earlier harvest can be beneficial for spring canola if a hard frost is expected. It is important to harvest at the optimum stage of ripening using the swathing and combining method to reduce green seed problems and seed shatter losses (NDSU, 2007). The quantity of green seed in the harvested crop affects quality and subsequently the market price of canola. Chlorophyll in seed is extracted with the oil during the crushing process and can increase the cost of refining the oil (Brown et al., 2008). Swathing can hasten the loss of green seed by about 2 days compared to seeds in standing crops (Brown et al., 2008). Direct combining is generally recommended for winter canola in the southern Great Plains region because dry-down is accelerated by higher air temperatures during the ripening stage and harvest period (Boyles et al., 2009). However, both methods have been used successfully for harvesting canola in this region. Swathing is generally recommended if harvest cannot be completed in a timely manner with direct combining.

Pushing, a new procedure being considered for harvesting canola, is reportedly a faster and less expensive alternative to swathing (Boyles et al., 2009). The pushing procedure pushes the stalks over, but does not cut or break the stalks off. When the canola matures, it is combined in the opposite direction of the pushing procedure. Experience with the pushing procedure is very limited at this time.

When direct combining, the ideal time to harvest is when the average seed moisture of canola is 8 to 10% and no green pods are visible on the plants (Boyles et al., 2009). Canola is an indeterminate crop and will retain a few immature pods and seed at harvest. Growers are advised not to delay harvest to allow these smaller immature pods and seeds to mature because waiting will result in the larger, higher yielding seed pods to shatter and reduce potential yield. To reduce the potential for pod shatter, growers can harvest at a slightly higher moisture (10% to 15%) and then artificially dry the seed.

The decision of when to swath is critical and is based on the correct seed maturity stage and favorable weather conditions (Brown et al., 2008). Canola seed color is a good indication of the maturity of the crop, and is the factor used to determine the time to swath. Growers must examine only the pods on main stem for seed color change. When the overall moisture content of seed from the total plant averages 30% to 35%, about 30% to 40% of the seeds in pods on the main stem will have changed color or started to change color (NDSU, 2005). In the case of canola, growers should try to avoid swathing when the air temperatures are above 82 °F particularly when humidity is low (Brown et al., 2008). These weather conditions can result in higher shatter losses and some immature green seed. Swathing in the evening or at night when air temperatures are cooler allows the seed to dry at a slower rate helping prevent green seed and low oil content. Swathing over-ripe canola (80 % seed color change) results in fluffy cut rows of canola that are more susceptible to blowing and increased shattering. Seed moisture content and green seed counts should be checked to determine the proper time to begin combining. Under normal weather conditions, this is generally 10 to 14 days after swathing for spring canola in North Dakota (NDSU, 2007) and 5 to 14 days for winter canola in the southern Great Plains region (Boyles et al., 2009).

Canola should be stored under cool, dry conditions. The optimum storage conditions for canola are 55°F and 7% seed moisture and the storage life of canola doubles with every 10° reduction below 77° and every 1% reduction in seed moisture below 9%. (Boyles et al., 2009). Canola seed is very sensitive to heating in storage (Brown et al., 2008) and storage bins must be properly aerated or vented for heat and moisture to escape.

VIII.D. Management of Insects

Insect pest infestations are a limiting factor in successful canola production (Brown et al., 2008). Insect pests can reach infestation levels that cause yield reductions and often a corresponding reduction in the oil content of canola. Insect pests can cause damage throughout the growing season (Boyles et al., 2009) on flowers, leaves, developing buds, seed pods, and developing seeds of canola. Seedling canola is especially vulnerable to chewing insects. A severe infestation of flea beetles can completely destroy a stand of canola seedlings. Late season insect pests can typically cause yield losses of 20 to 50% in spring canola when left uncontrolled (Brown et al., 2004). Some insects, such as aphids act as vectors for plant viruses like turnip mosaic, cauliflower mosaic virus, aster yellows and beet western yellows. Damage from insect pests is more severe during periods of stress, especially drought stress. Spring and winter canola varieties are impacted by the same insect pests. However, some pests negatively impact one variety

more than the other. Crop rotation, volunteer and wild mustard control, and managing crop residues are all important cultural practices for insect control.

The insect pests of spring and winter canola varieties in the U.S. are listed in Table VIII-4. Flea beetle is a serious insect pest in spring canola wherever it is grown (Brown et al., 2008). This insect pest is less important in winter canola, except where winter and spring canola are grown in the same area. The most significant plant injury and subsequent yield loss is caused by adult beetles in the early spring when the plants are in the cotyledon to two-leaf stage (Weiss et al., 2009). An insecticide seed treatment (imidacloprid or clothianidin) is advisable in spring canola to protect the seedlings (NDSU, 2005; 2007). Seed treatments usually provide at least 3 to 4 weeks of protection. If a seed treatment is not used or does not provide adequate protection, a foliar insecticide application is beneficial when 25% defoliation occurs on the cotyledons and true leaves (NDSU, 2005). Minimum or no-tillage systems generally have lower infestations of flea beetles (Weiss et al., 2009). In addition, planting early can allow establishment of the canola prior to beetle emergence.

Insect pests causing serious damage to winter canola include flea beetles, grasshoppers, army cutworms, diamondback moth larvae, aphids and maggots (Brown et al., 2008). Aphids have become the most important insect pest of canola in this region. The green peach and turnip aphid survive mild winters and increase to damaging levels during the early spring. Fields should be scouted from the seedling stage through pod development for damaging infestation levels of aphids. Aphid populations are sometimes high enough to cause significant stand reduction and yield losses (Brown et al., 2008). Predatory and parasitic insects are beneficial in controlling aphid populations. However, these insects alone will not prevent aphid populations from reaching damaging levels. An insecticide seed treatment (clothianidin, imidacloprid, or thiamethoxamare) is recommended in winter canola as a preventive management practice due to the frequency of damaging aphid populations in the fall in the southern Great Plains (Brown et al., 2008). Fields should be scouted in the spring for any subsequent build up of aphid populations.

Cabbage seedpod weevil is a major insect pest in winter canola in the Pacific Northwest (Brown et al., 2008). However, it has not been reported in the major canola production area of North Dakota and Minnesota (Weiss et al., 2009). Plant injury is caused by the adults feeding on buds. Yield losses of 20 to 40% are common from second generation feeding of cabbage seed pod weevil on the developing buds and seeds (Weiss et al., 2009). A foliar insecticide (bifenthrin or lambda-cyhalothrin) is recommended when three to four weevils are collected in a sampling sweep during scouting at bud stage.

Table VIII-4. Insect Pests by Canola Development Stage in Spring and Winter Canola in the U.S.[1]

Common Name	Latin Name
Seedling to Rosette Stage	
Crucifer and striped flea beetle	*Phyllotreta cruciferae, P. striolata*
Cutworm	*Euxoa species*
Diamondback moth	*Plutella xylostella*
Grasshopper	Various species
Green peach and turnip aphid	*Myzus persicae, Lipaphis erysimi*
Rosette to Flowering Stage	
Cutworm	
Diamondback moth	Plutella xylostella
Grasshopper	Various species
Green peach and turnip aphid	*M. persicae*, L. *erysimi*
Lygus bugs	*Lygus species*
Flowering to Pod Development	
Bertha armyworm	*Mamestra configurata*
Blister beetles	*Lytta nuttalli, Epicauta species*
Diamondback moth	*Plutella xylostella*
False chinch bug	*Nysius raphanus*
Grasshopper	Various species
Green peach, turnip and cabbage aphids	*M. persicae,* L. *erysimi, Brevicoryne brassicae*
Lygus bugs	*Lygus species*
Pod Development to Harvest	
Bertha armyworm	*Mamestra configurat*a
Cabbage aphid	*Brevicoryne brassicae*
Cabbage seedpod weevil	*Ceutorhynchus assimilis*
Crucifer and striped flea beetle	*Phyllotreta cruciferae, P. striolata*
False chinch bug	*Nysius raphanus*
Grasshopper	Various species
Harlequin bug	*Murgantia histrionica*
Lygus bugs	*Lygus species*

[1]Source: (Boyles et al., 2009; NDSU, 2005).

Other insect pests can reach populations that cause serious damage in spring and winter canola. Fields should be scouted throughout the growing season for economic threshold levels of insect pests. Economic threshold levels have been established for most insect pests by university entomologist to determine whether an insecticide treatment is justified. Foliar insecticides currently registered in the U.S. for use in canola include bifenthrin, deltamethrin, gammacyhalothrin, lamba cyhalothrin and methyl parathion (Brown et al., 2008). Insecticides were used only on approximately 3% of the canola acres in North Dakota in 2008 (Zollinger et al., 2009). Growers must be careful in the selection and application of insecticides. Most insecticides are non-selective and may damage non-pest insects. Bee keepers frequently set their hives close to flowering winter

canola fields because canola is an early and very rich source of pollen for bees (Brown et al., 2008). Therefore, growers need to exercise special care to protect honey bees from insecticide applications.

VIII.E. Management of Diseases and Other Pests

Plant diseases can be a serious problem in canola production. Diseases attack canola at all stages of development and can be soilborne, seedborne, or airborne, and also spread from infected crop residue (Boyles et al., 2009). Proper management of diseases is critical to maximize yields in canola.

Diseases that affect canola include blackleg (*Leptosphaeria maculans*), Sclerotinia stem rot (*Sclerotinia sclerotiorum*), Alternaria black spot (*Alternaria spp*.), downy mildew (*Peronospora parasitica*), powdery mildew (*Erysiphe cruciferarum*), black rot (*Xanthomonas campestris*) and aster yellow (Boyles et al., 2009; NDSU, 2005). In addition, there is a fungal seedling disease complex that includes *Pythium spp., Fusarium spp*., and *Rhizoctonia spp*. Blackleg and Sclerotinia stem rot are the most serious diseases in spring and winter canola.

Blackleg fungus is a seedborne disease that is commonly introduced by planting infected seeds (Brown et al., 2008). Once introduced into an area, it will remain as long as canola is grown there. The virulent or aggressive strains can infect early growth causing leaf spots any time from the seedling stage to maturity (NDSU, 2005). Seed pods and seeds also may be infected resulting in split pods and seed loss. Temperatures in the 70s and extended periods of wet plant canopy favor infection. Temperatures above 86°F and below 50°F inhibit development of the disease (NDSU, 2005). Several management practices are available to control or minimize this disease (NDSU, 2005). The most important practice is planting disease-free, certified seed where blackleg has not been introduced. Blackleg has yet to be introduced in the Pacific Northwest region and growers are advised to plant locally produced seed to prevent the introduction of the disease (Brown et al., 2008). A fungicide seed treatment should be used such as Helix Liteor Helix XTra which contains fungicides and an insecticide for flea beetle control. Growers should plant blackleg resistant varieties. Many of the *B. napus* canola varieties have moderate to good blackleg resistance. Deep tillage is recommended to bury the canola residue and speed decomposition of the residue and prevent the release of blackleg ascospores to nearby fields. Foliar applications of azoxystrobin fungicide are effective on blackleg.

Sclerotinia stem rot is present in most of the canola growing regions and has been the most serious disease of canola in North Dakota and Minnesota (NDSU, 2005). This disease has caused estimated losses up to 13% in North Dakota and Minnesota and yield reductions approaching 50% have been reported on seriously infected fields (NDSU, 2005). Canola primarily is susceptible during all bloom stages and shortly thereafter (NDSU, 2007). Wet weather immediately preceding and at flowering favors development of the Sclerotinia fungus. The first step in minimizing this disease is to plant canola in a four-year rotation with non-susceptible crops (Brown et al., 2008). The Sclerotinia fungus may survive four to six years in the soil (NDSU, 2005). Field peas and

flax are much less susceptible to the Sclerotinia fungus and are good rotational crops as are small grains and maize which are immune to the disease (NDSU, 2005). Sunflowers, dry beans, crambe, chickpeas, lentils and soybeans are some of the most susceptible host crops and should be avoided in the rotation. Many broadleaf weeds, including lambsquarters, Canada thistle, ragweed and marshelder are also susceptible host plants. All canola varieties are susceptible to Sclerotinia fungus, but some varieties are less susceptible than others and will perform better in moderate to severe disease pressure (NDSU, 2005). Foliar applications of a fungicide (bosclid or azoxystrobin) provide effective suppression of Sclerotinia stem rot. Since fungicides are expensive, applications should be limited to fields where: 1) the yield potential is above normal (2,000 lbs per acre or more); 2) weather preceding early bloom has been wet, 3) additional rain and high humidity is expected, and 4) Sclerotinia has been a problem in recent years (NDSU, 2005). Foliar fungicides were applied to approximately 19% of the canola acres in North Dakota in 2008 (Zollinger et al., 2009).

VIII.F. Weed Management

Weeds compete with canola for light, nutrients, and soil moisture and can be a major limiting factor in canola production. Canola yields can be reduced by as much as 50% due to weed competition with canola (CCC, 2006). The primary factors affecting the amount of yield loss are the weed species, weed density and the duration of the competition (CCC, 2006). Research studies have shown that weeds that emerge before or with the crop such as wild oats result in greater yield loss than weeds that emerge after the crop (CCC, 2006). Canola in the seedling stage is a poor competitor with weeds (NDSU, 2005). However, once established and with proper management, winter canola suppresses and out-competes most annual weeds with proper management (Boyles et al., 2009). Both Canada thistle and wild oats are very competitive with canola early in the growing season. Canada thistle is three or four times more competitive with canola than wild oats (CCC, 2006). Ten Canada thistle plants per square meter resulted in 10% yield loss while forty plants per square meter resulted in over 50% yield loss (CCC, 2006). A wild oat density of thirty plants per square meter caused between 15 and 20% yield loss (CCC, 2006). However, an average infestation of wild oats will range from 60 to 100 plants per square meter.

Knowing the critical period for weed control (CPWC) is useful in determining the need for and timing of herbicide applications (Knezevic et al., 2002). The CPWC is defined as the period in the crop growth cycle during which weeds must be controlled to prevent yield losses. Since the morphology, physiology, and development of each crop is different, the CPWC is likely to be unique for every crop.

Field studies conducted in 1998 and 1999 indicate that canola must be maintained weed-free up to the four-leaf stage (17 to 38 days after emergence) to consistently prevent a yield loss greater than 10% (Martin et al., 2001). However, the critical weed-free period is influenced by crop seeding date. Early-seeded canola may result in extending the CPWC to the six-leaf stage (41 days after emergence). Originally pre-emergent herbicide applications in canola provided weed control for the duration of this CPWC. With the advent of herbicide-tolerant canola varieties, post-emergent applications have been the

dominate method of herbicide application. The goal in timing post-emergent applications is to delay application for as long as practical to capture the most weed flushes without incurring yield loss. Frequently the crop canopy will prevent the establishment of late-emerging weeds and decrease the need for post-emergent herbicide applications (Martin et al., 2001). The same studies on CPWC determined that weeds need to be removed by the four-leaf stage of canola to prevent a yield loss of greater than 10%. Studies conducted in 1998 to 2000 at different locations with three different herbicide-tolerant canola systems generally confirmed these findings (Harker et al., 2006). Based on these studies, the first post-emergent applications would need to be applied prior to the four-leaf stage. Clayton et al., (2002) reported a yield benefit when removing weeds as early as the one- to two-leaf stage of canola in certain conditions. Although late-emerging weeds are not always competitive with the crop, controlling these weeds may be beneficial to reduce contaminants such as chaff, stems, weed seeds etc. that could lower the grade of the canola seed and prevent weeds from producing seed and infesting subsequent crops (Harker et al., 2006).

Crop rotations and environment have a significant impact on the adaptation and occurrence of weeds in canola. The most common weeds in canola for each growing region are presented in Table VIII-5, 6 and 7. Common weeds are those species which can be found abundantly infesting a significant portion of the acreage throughout the region. These weeds are most commonly present when no weed management intervention has occurred.

Table VIII-5. Common Weeds in Spring Canola Production in North Dakota[1]

Annual Grass Weeds	Annual Broadleaf Weeds	Biennial and Perennial Weeds
Foxtail, green and yellow	Buckwheat, wild	Quackgrass
Volunteer Cereals	Kochia	Thistle, Canada
Wild Oat	Field Pennycress	
	Lambsquarters, common	
	Mustard, wild	
	Pigweed species	
	Thistle, Russian	

[1]Source: (Zollinger, 2003; Jenks-NDSU Personal Communication, 8/2/2010).

Table VIII-6. Most Common Weeds in Winter Canola in the Great Plains[1]

Annual Grass Weeds	Annual Broadleaf Weeds	Winter Annuals
Japanese brome	Blue mustard	Henbit
Cheat	Bushy wallflower	Common chickweed
Downy brome	Wild mustard	Cheatgrass
Rescuegrass	Tumble mustard	Down bromegrass
Feral rye	Tansy mustard	Mustards
Jointed goatgrass	Flixweed	Volunteer cereals
Italian ryegrass	Field pennycrest	
Wild oat	Shepherd's purse	
Volunteer cereals		

[1]Source: (Boyles et al., 2009).

Table VIII-7. Most Common Weeds in Spring and Winter Canola in the Pacific Northwest[1]

Annual Grasses	Annual Broadleaf Weeds	Perennial Weeds
Downy brome	Field pennycress	Canada thistle
Wild oat	Catchweed bedstraw	
Italian ryegrass	Prickly lettuce	
Volunteer cereals	Chamomile mayweed	
Rattail fescue	Common lambsquarters	
	Redroot Pigweed	
	Russian thistle	
	Wild mustard	
	Tumble mustard	
	Birdsrape mustard	

[1]Source: (Davis – University of Idaho, Personal Communication, 7/30/2010; Wysocki – Oregon State University, Personal Communication, 7/30/2010).

Mechanical methods of weed control including tillage have been used for centuries to control weeds in crop production. Spring or fall pre-plant tillage can effectively reduce the competitive ability of weeds by burying the plants, disturbing or weakening the root

systems or causing sufficient physical injury to kill the plants. Additionally selective herbicides are an effective management tool to supplement cultural methods for controlling annual and perennial weeds in canola. The number of registered herbicides for canola in-crop application is relatively limited compared to the number available for use in other crops such as maize, soybean, and small grain cereals. Sethoxydim, clethodim, quizalofop and imazamox are post-emergence in-crop herbicides for control of annual grasses and broadleaf weeds in conventional canola varieties (NDSU, 2005). In addition, along with pre-plant incorporated herbicides ethalfluralin and trifluralin, glyphosate and glufosinate herbicides can be applied in conventional canola as a pre-plant burndown treatment for control of emerged weeds in no-tillage systems. Table VIII-8 provides a summary of the herbicides used in conventional and biotechnology-derived canola in North Dakota in 2008.

Table VIII-8. Herbicide Usage in Canola in North Dakota in 2008[1]

			Number of Applications	
Herbicide	**Acres Treated**	**% Acres Treated**	**1X**	**2X**
Clethodim	12,600	13.6	100.0	
Glufosinate	354,300	38.9	99.4	0.6
Glyphosate	660,300	72.6	58.0	42.1
Imazamox	21,200	2.3	48.4	51.6
Quizalofop	31,700	3.5	100.0	
Other herbicides	29,200	3.2	100.0	

[1]Source: (Zollinger et al., 2009).

Herbicide-tolerant canola was introduced to provide farmers with additional options to improve crop safety and/or improve weed control. The herbicide-tolerance traits enable the use of certain herbicides in canola that previously would not provide satisfactory crop safety when applied post-emergence to conventional canola. Roundup Ready, Liberty Link, and Clearfield-tolerant varieties allow the in-crop use of broad spectrum post-emergence herbicides, glyphosate, glufosinate, and imazamox, respectively. In 2008 herbicide-tolerant canola varieties were planted on approximately 97% of the canola acreage in North Dakota (Zollinger et al., 2009). Glyphosate-tolerant canola was commercially introduced in 1999. Roundup Ready canola is tolerant to post-emergence applications of glyphosate. Glyphosate was applied to approximately 73% of the canola acreage in 2008 which includes pre-plant burn down and in-crop applications (Table VIII-8). Liberty Link-tolerant canola was introduced in 1995 and is tolerant to post-emergence applications of glufosinate. Glufosinate is the second most commonly applied herbicide used in canola (38% of acres) (Table VIII-8). Clearfield varieties are tolerant to imazamox herbicide. Roundup Ready and Liberty Link are biotechnology derived traits while Clearfield was developed through traditional breeding techniques.

Tables VIII-9 and 10 provide a summary of the efficacy of herbicides on weed species commonly present in canola as well as their persistence in soil. Glyphosate is rated excellent on all but one annual grass (wild oat – good to excellent). Glufosinate and

imazamox are rated good and/or excellent on annual grasses, except for quackgrass where the rating is poor and fair, respectively. Glyphosate is the only herbicide in canola that is rated excellent on quackgrass, a perennial grass species. Glufosinate and imazamox are rated fair to excellent on all the annual and biennial broadleaf weeds with the exception of Canada thistle, buckwheat, mallow, ragweed and wormwood. The ratings for glyphosate are more variable on broadleaf weeds ranging from excellent to poor-excellent for all annual and biennial weeds and good to excellent on Canada thistle. It is important to note that glyphosate, glufosinate and imazamox, the three main herbicides used in herbicide-tolerant canola are rated as not persisting in soil (NDSU, 2005).

The benefits of growing herbicide-tolerant canola varieties include superior weed control, higher yield, less dependence on tillage and summer-fallow, and higher net return on profits (CCC, 2001; Harker et al., 2000). Roundup Ready and Liberty Link hybrid varieties are the dominate varieties in canola production with 56% and 39% of the acreage in North Dakota, respectively (Zollinger et al., 2009). The acreage planted with Clearfield varieties is only 1.9%. The superior weed control ratings presented for glyphosate and glufosinate in Tables VIII-9 and VIII-10 explain the reason for the success of the Roundup Ready and Liberty Link weed control systems (Harker et al., 2004). The consistency of monocot weed control was usually greater for the Roundup Ready system than for the Liberty Link or Clearfield systems. The researchers also concluded that the management risks were usually less apparent for the Roundup Ready system. The yield and performance of the canola variety is also an important factor in the selection of the herbicide-tolerant canola system. Determining the best combination of weed management and variety performance is important to the profitability and net returns in canola.

Current label directions indicate glyphosate agricultural herbicides can be applied post-emergence on glyphosate-tolerant canola from emergence to the 6-leaf stage of development in spring canola varieties. To prevent early weed competition and maximize yield potential, the first application of glyphosate to glyphosate-tolerant canola should be made at the two- to three-leaf stage. An additional application can be made up to the six-leaf stage to control any late-emerging weeds. Two applications of glyphosate are made on approximately 42% of the canola acreage (Table VIII-8). Most of the acres receiving two applications are no-till seeded acres where the first application applied is for burn down of emerged weeds and the second application is in-crop to control weeds emerging later.

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Table VIII-9. Grass Weed Species Responses and Persistence in Soil to Herbicides Applied in Canola Production[1]

Product	Barnyardgrass	Field Sandbur	Foxtail, Green	Foxtail, Yellow	Volunteer Cereals	Wild Oat	Wild Proso Millet	Quackgrass	Herbicide Persistence
Preplant Incorporated									
Ethalfluralin	E	G	E	E	G	F	P-F	N	S
Trifluralin	E	G	E	E	N	P-F	P-F	N	S
Postemergence									
Clopyralid	N	N	N	N	N	N	N	N	S
Imaxamox	E	F-G	E	G-E	G-E	E	G-E	F	N
Glyphosate	E	E	E	E	E	G-E	E	E	N
Glufosinate	E	G	E	G	F-G	G-E	E	P	N
Quizalofop	E	E	E	G-E	E	G-E	E	G-E	N
Sethoxydim	E	E	E	E	E	G-E	E	F	N
Clethodim	E	E	E	E	E	E	E	G	N

[1]Source: (NDSU, 2005). Weed control ratings based on the following scale: E = Excellent = 90 to 99% control, G = Good = 80 to 90% control, F = Fair = 65 to 80% control, P = Poor = 40 to 65 % control, and N = None = no control. Herbicide persistence ratings are for residues present 12 months after application: O = Often, S = Seldom, and N = None.

Table VIII-10. Broadleaf Weed Species Responses to Herbicides Applied in Canola Production[1]

Product	Buckwheat	Cocklebur	Flixweed	Kochia	Lambsquarters	Lanceleaf Sage	Mallow, Common	Mallow, Venice	Marshelder	Mustard, Wild	Nightshade, E. Black	Nightshade, Hairy	Redroot Pigweed	Prickly Lettuce	Ragweed, Common	Smartweed, Annual	Sunflower	Thistle, Russian	Wormwood, Biennial	Thistle, Canada
Preplant Incorporated Only																				
Ethalfluralin	P-F	P	P	F-G	E	N	-	F-G	N	N	P	P	E	P	P	P	N	G-E	N	N
Trifluralin	P-F	N	P	F	G-E	N	-	F-G	N	N	N	N	E	N	P	P	N	G	N	N
Postemergence																				
Clopyralid	F-G	E	N	N	P-F	F	P	P	E	N	E	E	P	E	G-E	G-E	G-E	P-F	E	E
Imaxamox	P	G-E	E	E	F	E	P	P	G-E	E	E	E	E	-	P	G-E	E	G-E	P	N-P
Glyphosate	F-G	E	G-E	F-E	P-E	E	F-G	E	G-E	G-E	F-G	F-G	E	E	E	F-E	G-E	G	G-E	G-E
Glufosinate	E	E	G-E	E	F-G	E	E	E	E	E	E	E	E	G-E	E	E	E	G-E	E	P
Quizalofop	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Sethoxydim	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Clethodim	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N

[1]Source: (NDSU, 2005). Weed control ratings based on the following scale: E = Excellent = 90 to 99% control, G = Good = 80 to 90% control, F = Fair = 65 to 80% control, P = Poor = 40 to 65 % control, and N = None = no control.
'-' = No data available

VIII.G. Second Generation Glyphosate-tolerant Canola – MON 88302

Monsanto Company has developed a second-generation glyphosate-tolerant canola product, MON 88302, designed to provide growers with improved weed control through greater flexibility of glyphosate herbicide application. MON 88302 produces the same 5-enolpyruvylshikimate-3-phosphate synthase (CP4 EPSPS) protein that is produced in commercial Roundup Ready crop products, via the incorporation of a *cp4 epsps* coding sequence. The CP4 EPSPS protein confers tolerance to the herbicide glyphosate, the active ingredient in the family of Roundup agricultural herbicides.

MON 88302 utilizes an improved promoter sequence to enhance CP4 EPSPS expression in male reproductive tissues, compared to the FMV 35S promoter used to drive CP4 EPSPS production in the first-generation product, Roundup Ready canola (RT73). Glyphosate is a systemic herbicide and is translocated in the plant, generally in the direction of strong source (*e.g.,* leaf) to rapidly developing, or sink tissue. Sink tissues, such as pollen, that accumulate glyphosate and that are deficient in CP4 EPSPS expression are considered to be at risk for glyphosate injury. By virtue of enhanced CP4 EPSPS expression in male reproductive tissues, MON 88302 provides tolerance to glyphosate during the sensitive reproductive stages of growth, and enables the application of glyphosate at later stages of development and at higher rates than is possible with the current product.

It is anticipated that canola production practices with MON 88302 will remain the same as they are for the first-generation Roundup Ready canola system. This includes the use of rotational crops, tillage systems, planting and harvesting operations and equipment. In addition to having similar agronomic practices, MON 88302 will continue to provide growers flexibility and simplicity in weed control, and allow them to realize the environmental benefits associated with the use of conservation-tillage and integrated weed management practices that are facilitated by the first-generation Roundup Ready canola system.

The current label directions for Roundup Ready spring canola allow for a single application of 0.39 to 0.56 lbs. of glyphosate a.e. per acre up to the 6-leaf growth stage. However, applications greater than 0.39 lbs a.e. per acre after the 4-leaf stage can result in crop injury. As an alternative, two sequential applications of up to 0.39 lbs. of glyphosate a.e. per acre may be applied up to the 6-leaf stage. Due to crop tolerance concerns, the total in-crop application of glyphosate cannot exceed 0.78 lbs. a.e. per acre. MON 88302 provides greater crop tolerance which will permit higher rates and a wider period for application of glyphosate in spring canola compared to the first-generation Roundup Ready canola system. Once the glyphosate label is amended by the EPA, MON 88302 will permit two in-crop sequential glyphosate applications up to 0.77 lbs. a.e. per acre each in spring canola or one application prior to the 6-leaf growth stage up to 1.55 lbs of glyphosate a.e. per acre. Total in-crop applications will be increased from 0.78 to 1.55 lbs of glyphosate a.e. per acre for spring canola.

In winter varieties of Roundup Ready canola, a single application up to 0.77 lbs of glyphosate a.e. per acre can be made in the fall up to the 6-leaf growth stage. However,

crop injury can result at application rates greater than 0.56 lbs a.e. per acre prior to the 6-leaf stage in the fall. A sequential application can be made the following spring up to 0.77 lbs a.e. per acre prior to the bolting stage. Two in-crop applications not to exceed a total of 1.55 lbs. a.e. per acre of glyphosate can be made in winter varieties of the first generation Roundup Ready canola. Recommended application rates for glyphosate in winter canola will likely remain unchanged with the introduction of MON 88302.

However application timing will be extended up to the first flower stage for both spring and winter varieties with MON 88302. The higher glyphosate rates and extended timing for applications will provide improved control of weed species such as Canada thistle, dandelion, sow thistle, common lambsquarters, kochia, smartweed and wild buckwheat. The increased maximum rate limit in spring canola will provide greater flexibility to utilize the appropriate glyphosate rate for difficult to control weed species when making sequential application of glyphosate

Weed competition can be a major limiting factor in canola production leading to significant yield reductions (CCC, 2006). Certain perennial weeds, such as Canada thistle, are known to be particularly important to control in canola production. For example, studies have demonstrated that only 10 Canada thistle plants per square meter have resulted in 10% yield loss while 40 plants per square meter have resulted in over 50% yield loss (CCC, 2006). Glyphosate is highly effective against the majority of annual and perennial grasses and broad-leaf weeds including Canada thistle (NDSU, 2005; 2010; Padgette et al., 1996). Glyphosate has been shown to have a favorable safety profile by the U.S. EPA (1993b) which has concluded that use of glyphosate will not pose unreasonable risks to human or the environment.

VIII.H. Crop Rotation Practices in Canola

The well-established farming practice of crop rotation is a key management tool for growers. One of the main purposes of growing canola in rotation with other crops is to mitigate or break canola disease and insect cycles (Brown et al., 2008; NDSU, 2005). Including canola in wheat rotations has resulted in 10% to 20% yield increases in wheat compared to continuous wheat (Brown et al., 2008). Incorporating canola in small grain rotations has also decreased disease incidence, increased quality of cereal crops and provides better herbicide options for controlling certain weeds such as downy brome, jointed goat grass, wild oat, Italian ryegrass and feral rye, which are difficult to control in small grains (Brown et al., 2008). Although the benefits of crop rotations can be substantial, the grower still must make cropping decisions based on both the agronomic and economic returns of various cropping systems. Crop rotations also afford the grower the opportunity to diversify farm production in order to minimize market risks.

Growers should not plant canola following canola to avoid a buildup of soilborne diseases like Sclerotinia stem rot, blackleg or club root (Brown et al., 2008). Where fungal diseases are present, canola should be planted only once every four years. In addition, growers should avoid planting canola after other crops like legumes (pea, lentil chickpea, soybean, field and dry been) or sunflower that are susceptible to Sclerotinia,

Rhizoctonia and Fusarium root rots. Canola is most commonly grown following small grain cereals such as winter or spring wheat, barley, maize or sorghum (Brown et al., 2008).

Crop rotations with canola may change due to economic conditions and market opportunities. Agronomic practices such as rotations for canola vary from region to region. This section provides a detailed description and quantification of the rotational cropping practices immediately following canola, by state, that account for 99+% of the total canola acreage. These data are presented in Table VIII-11.

In the northern Great Plains (North Dakota and Minnesota), canola is generally grown in a 3- or 4-year rotation with small grains (wheat, barley, oats) (Hoefing – North Dakota State University, Minot - Personal Communication, 8/2/2010; Miller – Montana State University – Personal Communication, 8/27/2010). However, flax, soybean, dry beans, dry peas and sunflower could also be added into the rotation on occasion. A typical 4-year rotation sequence could be; canola-wheat-flax-barley with oat, soybean or dry beans substituted for flax. Barley could also be replaced with wheat depending on economic conditions. A typical 3-year rotation sequence could be canola-wheat-wheat with barley replacing a wheat crop on occasion.

In Oklahoma, approximately 95% of the winter canola is grown in a 3-year rotation with winter wheat (wheat-canola-wheat) (Peeper, Oklahoma State University – Personal Communication, 8/3/2010). Soybean or sorghum may follow canola in place of winter wheat in a 3-year rotation.

In the Pacific Northwest states (Idaho and Oregon), spring canola is generally grown in a 3-year rotation with wheat or barley – spring canola- winter wheat-spring cereal (wheat or barley) (Davis - University of Idaho; Wysocki – Oregon State University - Personal Communication, 7/29/2010). The rotation sequences in winter canola could be as follows: winter canola-winter wheat-spring cereal-fallow or winter canola-fallow-winter wheat-fallow. Spring and winter canola is almost always followed with wheat.

The majority of the U.S. canola acreage (81%) is rotated to wheat (Table VIII-11). The second largest rotational crop following canola is barley (14%). The remaining canola acres, approximately 5%, are rotated to dry bean, field bean, flax, oats, sorghum, soybean, and sunflower.

Column J of Table VIII-11provides the acreage of canola in each state as a percentage of the total rotational crop acreage to indicate the level that canola is the primary crop preceding rotational crops. For the U.S., (Table VIII-11), this percentage is 0.5%. The percentage of canola preceding each state's rotational crop acreage ranges from 0.1% (MN and MT) to 4.6% (ND) (Table VIII-11).

Table VIII-11 also provides an assessment of the Roundup Ready canola acreage that will be rotated to another Roundup Ready crop in the U.S. Roundup Ready soybean is the only Roundup Ready crop rotated with canola and it has been deregulated by the USDA. For the purposes of this assessment, the adoption rates used for Roundup Ready

soybean in 2010 were obtained from the USDA-NASS Acreage report - June 2010 (USDA-NASS, 2010a). The percentage of the total rotational crop acreage rotated from Roundup Ready canola to another Roundup Ready crop (Table VIII-11 - Column K) is 0.0005% for the U.S. and ranges from none (ID, MT, OR) to 0.04% (ND) across the canola growing states.

Table VIII-11. Rotational Practices in the U.S. Following Canola Production

A	B	C	D	E	F	G	H	I	J	K
State	**Total Canola Acres[1]**	**Major Crops That Follow Canola In Rotation**	**Total Acreage of Rotation Crop in States[1]**	**Percent of Rotational Crop Rotated From Canola[2]**	**Rotational Crop Acres Following Canola[3]**	**Percent Rotational Crop of Total Canola[4]**	**Percent Roundup Ready Rotational Crop Option[5]**	**Acreage of Roundup Ready Rotational Crop Option[6]**	**Percent of Canola Acres Preceding Major Rotations[7]**	**Estimated Percentage of Roundup Ready Crops as Major Rotations[8]**
United States	**811**	Barley	3567	3.2	113	13.9	NA			
		Dry Bean	654	1.1	7	0.9	NA			
		Field Pea	863	0.02	0.2	0.02	NA			
		Flax	317	2.2	7	0.9	NA			
		Oats	3404	0.2	7	0.9	NA			
		Sorghum	6633	0.02	1	0.1	NA			
		Soybean	77451	0.01	8	1.0	93%	7.4		
		Sunflower	2030	0.3	7	0.9	NA			
		Wheat	59133	1.1	659	81.3	NA			
			Total: 154,369		**Total: 809**			**Total: 7.4**	**0.5%**	**0.0005%**
ID	**15**	Wheat	1,310	1.1	15	100	NA	0		
			Total: 1,310		**Total: 15**			**Total: 0**	**1.1%**	**0%**
MN	**13**	Barley	95	2.1	2	15	NA			
		Dry Bean	68	0.2	0.1	1	NA			
		Flax	3	3.3	0.1	1	NA			
		Oats	250	0.04	0.1	1	NA			
		Soybean	7200	0.001	0.1	1	93%	0.1		
		Sunflower	45	0.2	0.1	1	NA			
		Wheat	1655	0.6	10	80	NA			
			Total: 9,316		**Total: 13**			**Total: 0.1**	**0.1%**	**0.001%**
MT	**6.5**	Barley	870	0.1	1	15	NA			
		Field Pea	240	0.04	0.1	2	NA			
		Wheat	5520	0.1	5	83	NA			
			Total: 6,630		**Total: 6.5**			**Total: 0**	**0.1%**	**0%**

Table VIII-11. Rotational Practices in the U.S. Following Canola Production (continued)

A	B	C	D	E	F	G	H	I	J	K
State	Total Canola Acres[1]	Major Crops That Follow Canola In Rotation	Total Acreage of Rotation Crop in States[1]	Percent of Rotational Crop Rotated From Canola[2]	Rotational Crop Acres Following Canola[3]	Percent Rotational Crop of Total Canola[4]	Percent Roundup Ready Rotational Crop Option[5]	Acreage of Roundup Ready Rotational Crop Option[6]	Percent of Canola Acres Preceding Major Rotations[7]	Estimated Percentage of Roundup Ready Crops as Major Rotations[8]
ND	**730**	Barley	1210	8.3	110	15	NA			
		Dry Bean	533	1.3	7	1	NA			
		Flax	295	2.4	7	1	NA			
		Oats	350	2	7	1	NA			
		Soybean	3900	0.2	7	1	94%	6.6		
		Sunflower	770	0.9	7	1	NA			
		Wheat	8680	6.7	584	80	NA			
			Total: 15,738		**Total: 730**			**Total: 6.6**	**4.6%**	**0.04%**
OK	**42**	Sorghum	250	0.4	1	2.5	NA			
		Soybean	405	0.3	1	2.5	90%	0.9		
		Wheat	5700	0.7	40	95	NA			
			Total: 6355		**Total: 42**			**Total: 0.9**	**0.7**	**0.01%**
OR	**4.9**	Wheat	890	0.6	4.9	100	NA	0		
			Total: 890		**Total: 4.9**			**Total: 0**	**0.6%**	**0%**

The United States summary was developed by compiling the data from all states. All acreages are expressed as 1000s of acres. Unlike the individual state data, the data in Column G for the US were obtained by dividing Column F by Column B. NA denotes not applicable.

[1] Acreage planted of the specific crops is based on 2009 planting data from the USDA-NASS, Crop Production - 2009 Summary, January 2010 (2010b).

[2] Column E obtained by dividing Column F by Column D and multiplying by 100.

[3] Column F obtained by multiplying Column B by Column G.

[4] Rotational crop percentages based on estimates from personal communications with individual state canola Extension Crop Production Specialists (Hoefing, North Dakota State University, 8/2/2010; Peeper, Oklahoma State University, 8/3/2010; Davis, University of Idaho, 7/29/2010; Wysocki, Oregon State University, 7/29/2010).

[5] Roundup Ready rotational crop adoption rates for soybean are based on 2010 planting data taken from USDA-NASS, Acreage Report, June 2010 (2010a).

[6] Column I obtained by multiplying Column F by Column H except as noted above for U.S.

[7] Column J obtained by dividing Column B by Column D Total and multiplying by 100.

[8] Column K obtained by dividing Column I Total by Column D Total and multiplying by 100.

VIII.I. Canola Volunteer Management

Volunteer canola is defined as a plant that has germinated and emerged unintentionally during the production of a subsequent rotational crop. Canola seeds can remain in a field after canola harvest as a result of pods shattering before or at harvest time. Canola seeds can also remain in a field when pod placement on the plants is too close to the ground for the combine head to collect all the pods, or the combine is improperly adjusted for efficient harvesting. Canola seeds can also be transported to other fields with the harvested equipment. Volunteer canola will compete with the succeeding rotational crop and may affect yield depending on the volunteer density (NDSU, 2005). Although canola can volunteer for several years following a canola crop, management problems with volunteer canola are not common (Boyles et al., 2009). In spring canola, seeds that remain on or near the soil surface may germinate in the fall and be killed by frost (NDSU, 2005). In winter canola, the seed typically germinates after summer rains and can be controlled by tillage. A Canadian study indicates that the incidence of fields with canola volunteers in the subsequent rotational crop is about 10% (CCC, 2005).

Precautions need to be taken to minimize canola seed losses during swathing and combining operations. With spring canola, time should be allowed in the spring for the seeds to germinate in order to control the volunteers with tillage prior to planting. With no-till small grains, growers should add a labeled herbicide (2,4-D) to the glyphosate pre-emergence application for control of glyphosate-resistant canola volunteer plants. Canola volunteers will be controlled best when the herbicide is applied by the 5-leaf stage (NDSU, 2005). Canola volunteers become much more difficult to control with herbicides when they reach the 6-leaf to bolting stage. Table VIII-12 provides a listing of labeled herbicides that control volunteer glyphosate-tolerant canola in the various rotational crops, and their effectiveness.

No changes are anticipated from the introduction of MON 88302 on current volunteer management practices in the cultivation of commercial canola.

Table VIII-12. Ratings for Post-emergence Control of Volunteer Canola in Labeled Rotational Crops[1]

Product	Rate Product/Acre	Preemergence	Canola 3-leaf Stage	Canola 6-leaf Stage
Maize				
Accent	0.5 oz	-	E	E
Balance Pro	3 fl oz	E	-	-
Callisto	3 fl oz	-	E	G
Option	1.5 oz	-	E	E
Steadfast	0.75 oz	-	E	E
Soybean				
Extreme	1.5 pt	E	-	-
Flexstar	0.75 pt	-	E	E
Raptor	4 fl oz	-	E	G-E
Valor	2.5 oz	E	-	-
Dry Pea				
Basagran	0.5 pt	-	G-E	F
MCPA amine	0.5 pt	-	G-E	P
Pursuit	0.72 oz	G-E	G-E	G
Raptor	4 fl oz	-	E	G-E
Sencor	4 oz	E	-	-
Sunflower				
Assert	0.8 pt	-	E	G
Spartan	4 oz	P-F	-	-
Flax				
Bronate Advanced	0.8 pt	-	E	F-G
MCPA ester	0.5 pt	-	E	F-G
Spartan	4 oz	P-F	-	-
Wheat				
Bronate Advanced	0.8 pt	-	E	F-G
2,4-D ester	0.5 pt	-	G-E	P-G
Express	0.167 oz	-	E	G-E
Harmony GT	0.3 oz	-	F-E	P-F
MCPA ester	0.5 pt	-	G-E	F-G
Finesse[2]	0.3 oz		G-E	
Amber[2]	0.56 oz		G-E	
Agility[2]	3.2 oz		G-E	

[1] Sources: Modified from NDSU (2005). Weed control ratings: E = Excellent (90 to 99% control), G = Good (80 to 90% control), F = Fair (65 to 80 control), and P = Poor (40 to 65% control).
[2] Source: (Boyles et al., 2009). Control rating in winter wheat applied in fall or spring.

VIII.J. Weed Resistance to Glyphosate Herbicide

The risk of weeds developing resistance and the potential impact of resistance on the usefulness of a herbicide vary greatly across different modes of action, and is dependent on a combination of different factors. Monsanto considers product stewardship to be a fundamental component of customer service and business practices, and invests considerably in research to understand the proper uses and stewardship of our herbicide-tolerant soybean systems. This research includes an evaluation of the factors that can contribute to the development of weed resistance. Detailed information regarding herbicide resistance is presented in Appendix J.

VIII.K. Stewardship of MON 88302

Monsanto Company develops effective products and technologies and is committed to assuring that its products and technologies are safe and environmentally responsible. Monsanto demonstrates this commitment by implementing product stewardship processes throughout the lifecycle of a product and by participation in the Excellence Through Stewardship^{SM} (ETS) Program. These policies and practices include rigorous field compliance and quality management systems and verification through auditing. Monsanto's Stewardship Principles are also articulated in Technology Use Guides that are distributed annually to growers who utilize Monsanto branded traits.

As an integral action of fulfilling this stewardship commitment, Monsanto will seek biotechnology regulatory approvals for MON 88302 in all key canola import countries with a functioning regulatory system to assure global compliance and support the flow of international trade. These actions will be consistent with the Biotechnology Industry Organization (BIO) Policy on Product Launch. Monsanto continues to monitor other countries that are key importers of canola from the U.S. for the development of formal biotechnology approval processes. If new functioning regulatory processes are developed, Monsanto will make appropriate and timely regulatory submissions.

Monsanto is committed to utilizing the best industry practices on seed quality assurance and control to ensure the purity and integrity of MON 88302 seed. As with all of Monsanto's products, before commercializing MON 88302 in any country, a MON 88302 detection method will be made available to canola producers, processors and buyers.

Glyphosate is a non-selective herbicide registered with the U.S. EPA for the post-emergence control of annual and perennial grasses and broadleaf weeds in agriculture. Glyphosate has a long history of effective use in U.S. crop production, including maize, soybean, small grains, sorghum, cotton and canola production. Monsanto is seeking regulatory approvals with the U.S. EPA for an amended label for use of glyphosate on MON 88302 which incorporates higher application rates and later timing for applications. Although the amended labeling increases the rate of application and widens the application period relative to canola development, this use of glyphosate does not present any new environmental exposure scenarios. Glyphosate residue levels for this product

fall within the existing U.S. and Codex Maximum Residue Level of 20 ppm (Codex, 2011).

As with all U.S. EPA registered herbicides for agricultural use, it is possible that offsite movement during and/or following application can occur such that non-target plants may be exposed to direct spray or to spray drift. Potential impacts from drift have been mitigated through specific label use restrictions for glyphosate when applied with aerial equipment. Good management practices for application of all pesticides are also promoted by state university extension services (Jordan et al., 2009; University of Illinois, 2010).

VIII.L. Impact of the Introduction of MON 88302 on Agricultural Practices

Canola fields are typically highly managed agricultural areas that are dedicated to crop production. MON 88302 is likely to be used in common rotations on land previously used for agricultural purposes. Certified seed production will continue to use well-established industry practices to deliver high quality seed containing MON 88302 to growers. Cultivation of MON 88302 is not expected to differ from current canola cultivation using first-generation glyphosate-tolerant canola, with the exception of an opportunity to use glyphosate during an expanded window of application and at rates higher than those currently recommended and authorized for the first-generation product.

MON 88302 is similar to conventional canola in its agronomic, phenotypic, environmental, and compositional characteristics and has levels of tolerance to insects and diseases comparable to conventional canola (Sections V, VI and VII). Therefore, no significant impacts on current cultivation and management practices for canola are expected following the introduction of MON 88302. Based on this assessment, the introduction of MON 88302 will not impact current U.S. canola cultivation practices or weed management practices, other than intended weed control benefits.

IX. ENVIRONMENTAL CONSEQUENCES

IX.A. Introduction

This section provides a brief review and assessment of the plant pest potential of MON 88302 and its impact on agronomic practices as well as the environmental impact of the introduced CP4 EPSPS protein. USDA-APHIS has responsibility, under the Plant Protection Act (PPA) (7 U.S.C. § 7701-7772), to prevent the introduction and dissemination of plant pests into the U.S. Regulation 7 CFR § 340.6 provides that an applicant may petition APHIS to evaluate submitted data to determine that a particular regulated article does not present a plant pest risk and should no longer be regulated. If APHIS determines that the regulated article does not present a plant pest risk, the petition is granted, thereby allowing unrestricted introduction of the article.

According to the PPA, the definition of "plant pest" includes living organisms that can directly or indirectly injure, damage, or cause disease in any plant or plant product (7 U.S.C. § 7702[14]).

The regulatory endpoint under the PPA for biotechnology-derived crop products is not zero risk, but rather a determination that deregulation of the article in question is not likely to pose a plant pest risk. Information in this petition related to plant pest risk characteristics includes: 1) mode of action and changes to plant metabolism; 2) composition; 3) expression and characteristics of the gene product; 4) potential for weediness of the regulated article; 5) impacts to NTOs; 6) disease and pest susceptibilities; 7) impacts on agronomic practices; and 8) impacts on the weediness of any other plant with which it can interbreed, as well as the potential for gene flow.

The following lines of evidence form the basis for the plant pest risk assessment in this petition: 1) insertion of a single functional copy of the *cp4 epsps* expression cassette; 2) characterization of the CP4 EPSPS protein expressed in MON 88302; 3) safety and mode of action of the CP4 EPSPS protein; 4) compositional equivalence of MON 88302 seed to conventional canola; 5) phenotypic and agronomic characteristics demonstrating no increased plant pest potential including disease and pest susceptibilities; 6) negligible risk to non target organisms (NTOs) and threatened or endangered species (TES); 7) familiarity with canola as a cultivated crop; and 8) no greater likelihood to significantly impact agronomic practices, including land use, cultivation practices, or the management of weeds, diseases, and insects compared to commercially grown canola.

Using the assessment above, the data and analysis presented in this petition leads to a conclusion that MON 88302 is unlikely to be a plant pest, and therefore should no longer be subject to regulation under 7 CFR § 340.

In 2008, APHIS proposed amendments to 7 CFR § 340 that included provisions to utilize its noxious weed authority in regulating genetically engineered plants (73 FR 600008). Because the data presented in this petition demonstrate that MON 88302 has no potential to cause injury or damage to protected interests under the noxious weed authority,

MON 88302 would not be considered a “noxious weed” as defined by the Plant Protection Act.

IX.B. Plant Pest Assessment of MON 88302 Insert and Expressed Protein

This section summarizes the details of the genetic insert, characteristics of the genetic modification, and safety and expression of the CP4 EPSPS protein expressed in MON 88302 used to evaluate the food, feed, and environmental safety of MON 88302.

IX.B.1. Characteristics of the Genetic Insert and Expressed Protein

IX.B.1.1. Genetic Insert

MON 88302 was developed through *Agrobacterium*-mediated transformation of canola hypocotyls utilizing the plasmid vector PV-BNHT2672. PV-BNHT2672 contains one transfer DNA (T-DNA) that is delineated by Left Border and Right Border regions. The T-DNA contains the *cp4 epsps* coding sequence under the control of the *FMV/Tsf1* chimeric promoter, the *Tsf1* leader and intron sequences, and the *E9* 3′ untranslated region. The chloroplast transit peptide CTP2 directs transport of the CP4 EPSPS protein to the chloroplast and is derived from *CTP2* target sequence of the *Arabidopsis thaliana shkG* gene (Figure III-1; Table III-1). The characterization of the genetic modification demonstrated that a single copy of the T-DNA was inserted at a single locus of the genome, and no additional genetic elements, including backbone sequences, from PV-BNHT2672 were detected in MON 88302 (Figures IV-2 to IV-6). DNA sequence analyses confirmed integrity of the inserted *cp4 epsps* expression cassette and identified the 5’ and 3’ insert-to-genomic DNA junctions.

Additional sequence analysis of conventional canola genomic DNA confirmed a 9 base pair insertion immediately adjacent to the 3' end of the MON 88302 insert and a 29 base pair deletion immediately adjacent to the 5' end of the MON 88302 from the conventional genomic DNA. Those changes most likely occurred in MON 88302 upon insertion of the T-DNA. Such changes are quite common during plant transformation; these changes presumably resulted from double-stranded break repair mechanisms in the plant during the *Agrobacterium*-mediated transformation process (Salomon and Puchta, 1998). Finally, Southern blot analysis demonstrated that the DNA fingerprint of the T-DNA insert in MON 88302 has been maintained through multiple generations of breeding, thereby confirming the stability of the insert in multiple generations.

These data demonstrate that there are no unintended changes in the MON 88302 genome as a result of the insertion of the *cp4 epsps* expression cassette.

IX.B.1.2. Mode of Action

MON 88302 produces the same 5-enolpyruvylshikimate-3-phosphate synthase (CP4 EPSPS) protein that is produced in commercial Roundup Ready crop products, including the first generation product Roundup Ready canola, which confers tolerance to glyphosate. In most plants, glyphosate binds to the endogenous EPSPS enzyme and blocks the biosynthesis of EPSP thereby depriving the plant of essential amino acids

(Steinrücken and Amrhein, 1980). In Roundup Ready plants, requirements for aromatic amino acids and other metabolites are met by the continued action of the CP4 EPSPS enzyme in the presence of glyphosate (Padgette et al., 1996).

IX.B.1.3. Expressed Protein Safety

Several Roundup Ready crops that produce the CP4 EPSPS protein have been deregulated by the USDA and been reviewed during the biotechnology consultation process by the FDA. The CP4 EPSPS protein expressed in MON 88302 is identical to the CP4 EPSPS proteins in other Roundup Ready crops including Roundup Ready soybean, Roundup Ready 2 Yield soybean, Roundup Ready corn 2, Roundup Ready canola, Roundup Ready sugar beet, Roundup Ready cotton, Roundup Ready Flex cotton and Roundup Ready alfalfa. The safety of CP4 EPSPS proteins present in such biotechnology-derived crops has been extensively assessed (Harrison et al., 1996). Results from the protein characterization studies included in this petition confirmed the identity of the MON 88302-produced CP4 EPSPS protein and established the equivalence of MON 88302-produced protein to the *E. coli*-produced CP4 EPSPS protein (Section V.B.) used previously to demonstrate the safety of the CP4 EPSPS protein produced in other Roundup Ready crops. The Environmental Protection Agency (EPA) also reviewed the safety of the CP4 EPSPS protein and has established a tolerance exemption for the protein and the genetic material necessary for its production either in or on all raw agricultural commodities (U.S. EPA, 1996a). This exemption was based on a safety assessment that included rapid digestion in simulated gastric fluids, lack of homology to known toxins and allergens, and lack of toxicity in an acute oral mouse gavage study. A history of safe use is supported by the lack of any documented reports of adverse effects since the introduction of Roundup Ready crops in 1996. Therefore, it is concluded that the CP4 EPSPS protein poses no risk to human or animal health.

IX.B.1.4. Protein Expression Levels

CP4 EPSPS protein levels in MON 88302 ranged from 22 to 500 µg/g dw in root, forage, harvested seed and over-season leaf (Section V.C.). The levels of CP4 EPSPS from all tissues tested and across all sites are comparable to other commercialized Roundup Ready crops containing the CP4 EPSPS protein that have been cleared for environmental release by regulatory agencies around the world.

IX.B.2. Compositional Characteristics

Compositional analyses were conducted as recommended for canola seed (OECD, 2001) to assess whether levels of key nutrients, toxicants, and anti-nutrients in MON 88302 were equivalent to levels in the conventional control and to the composition of commercial reference varieties (Section VI). The background genetics of the conventional control (Ebony) were similar to that of MON 88302, but did not contain the *cp4 epsps* expression cassette. Seed was harvested from five individual sites in which MON 88302, the conventional control, and a range of commercial reference varieties were grown concurrently in the same field trial. The commercial reference varieties were used to calculate a 99% tolerance interval for each compositional component to define

the natural variability of each component in canola varieties that have a history of safe consumption.

Nutrients assessed in this analysis included proximates (ash, carbohydrates by calculation, moisture, protein, and total fat), fibers (acid detergent fiber [ADF], neutral detergent fiber [NDF], and total dietary fiber [TDF]), amino acids (18 components), fatty acids (FA; C8-C24,), vitamin E (α-tocopherol), and minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc) in seed. The toxicants assessed in seed included erucic acid and glucosinolates (alkyl glucosinolates [including 3-butenyl, 4-pentenyl, 2-hydroxy-3-butenyl, and 2-hydroxy-4-pentenyl glucosinolates], indolyl glucosinolates [including 3-indolylmethyl and 4-hydroxy-3-indoylmethyl], and total glucosinolates). The anti-nutrients assessed in seed included phytic acid and sinapine (as sinapic acid). All components analyzed in MON 88302 seed were either not statistically significantly different ($\alpha = 0.05$) compared to the conventional control, or, if significantly different, were within the natural variability of canola composition as expressed in the 99% tolerance interval. Collectively, the compositional analyses data support the conclusion that MON 88302 is as safe as and compositionally equivalent to conventional canola.

IX.B 3. Phenotypic and Agronomic and Environmental Interaction Characteristics

An extensive and robust set of information and data were used to assess whether the introduction of the glyphosate-tolerance trait altered the plant pest potential of MON 88302 compared to the conventional control. Phenotypic, agronomic, and environmental interaction characteristics of MON 88302 were evaluated and compared to those of the conventional control and considered within the variation among commercial reference varieties. These assessments included five general data categories: 1) germination, dormancy, and emergence; 2) vegetative growth; 3) reproductive growth (including pollen characteristics); 4) seed retention on the plant and lodging; and 5) plant response to abiotic stress and interactions with diseases and arthropods. Results from the phenotypic, agronomic, and environmental interaction assessment demonstrate that MON 88302 does not possess: a) increased weediness characteristics; b) increased susceptibility or tolerance to specific abiotic stresses, diseases or arthropods; or c) characteristics that would confer a plant pest risk or a significant environmental impact compared to the conventional control. Taken together, the results of the analysis support a determination that MON 88302 is no more likely to pose a plant pest risk or have a biologically meaningful change in environmental impact than conventional canola.

IX.B.3.1. Seed Dormancy and Germination

Seed germination and dormancy mechanisms vary with species and their genetic basis tends to be complex. Seed dormancy is an important characteristic that is often associated with plants that are considered weeds (Anderson, 1996; Lingenfelter and Hartwig, 2007). There were no changes in the dormancy or germination characteristics that are indicative of increased plant weediness or pest potential of MON 88302 compared to the conventional control (Section VII.C.1.).

IX.B.3.2. Plant Growth and Development

Evaluations of plant growth and development characteristics in the field are useful for assessing potential weediness characteristics such as lodging and pod shattering (Section VII.C.2.). Phenotypic characteristics such as early stand count, seedling vigor, days to first flowering, lodging, plant height, visual rating for pod shattering, quantitative pod shattering, seed maturity, seed quality, yield, seed moisture, and final stand count were assessed. Of the growth and development characteristics assessed between MON 88302 and the conventional control, no statistically significant differences were detected at a 5% level of significance ($\alpha = 0.05$) with the exception of days to first flowering and harvested seed moisture in a combined-site analysis of the data. The mean values of MON 88302 were within the natural variability of the commercial reference varieties for these characteristics. Therefore, the differences in these parameters are not considered to be biologically meaningful in terms of increased weediness or plant pest potential of MON 88302 compared to conventional canola.

IX.B.3.3. Response to Abiotic Stressors

No biologically meaningful differences were observed during comparative field observations between MON 88302 and the conventional control and their response to abiotic stressors, such as drought, wind, nutrient deficiency, etc. (Section VII.C.2.). The lack of significant biological differences in plant responses to abiotic stress support the conclusion that the introduction of the glyphosate-tolerance trait is unlikely to result in increased plant pest potential or an adverse environmental impact from MON 88302 compared to conventional canola.

IX.B.3.4. Pollen Morphology and Viability

Evaluations of pollen morphology and viability of non-glyphosate treated plants provide information useful in a plant pest assessment as it relates to the potential for gene flow to, and introgression of, the biotechnology-derived trait into other canola varieties and wild relatives (Section VII.C.3.). Pollen morphology and viability evaluations demonstrated no statistically significant differences ($\alpha = 0.05$) between MON 88302 and the conventional control. Taken together, these comparative assessments indicate that MON 88302 is not fundamentally different from conventional canola or more or less likely to have increased weediness or plant pest potential.

IX.B.3.5. Interactions with Non-target Organisms Including Threatened and Endangered Species

Evaluation of MON 88302 for potential adverse impacts on non target organisms (NTOs) is a component of the plant pest risk assessment. The safety of CP4 EPSPS proteins present in biotechnology-derived crops has been extensively assessed (Harrison et al., 1996; ILSI-CERA, 2010). A mouse gavage study demonstrated no acute oral toxicity (Harrison et al., 1996), and consequently the low potential for impact to terrestrial vertebrate NTOs including threatened and endangered vertebrate species. Impacts of deregulation of MON 88302 to NTOs and threatened and endangered species are

discussed in greater detail in Appendix I. Data from the 2009 North American phenotypic and agronomic study including observational data on environmental interactions such as plant-disease interaction, arthropod damage and arthropod abundance, were collected at select sites for MON 88302 and the conventional control (Section VII.C.2.; Appendix G). These results support the conclusion of no adverse environmental impact from cultivation of MON 88302 to non-target organisms and no increased incidence of disease in MON 88302. Additionally, after numerous environmental risk assessments on a range of plant species expressing the CP4 EPSPS protein, data indicate no correlation between CP4 EPSPS protein expression and any increased tendency for persistence or spread in the environment, alterations in reproductive biology affecting gene flow, or negative impacts on other organisms in the environment (ILSI-CERA, 2010).

MON 88302 produces the identical CP4 EPSPS protein as is found in commercial Roundup Ready crop products including Roundup Ready canola (RT73). This protein confers tolerance to glyphosate. The CP4 EPSPS protein is a member of the larger family of EPSPS proteins that are ubiquitous in plants and microbes in the environment, and have been isolated from both sources (Harrison et al., 1996; Klee et al., 1987; Schönbrunn et al., 2001; Steinrücken and Amrhein, 1984). The CP4 EPSPS protein is an enzyme in the shikimate metabolic pathway in plants and microorganisms and is important in the production of aromatic amino acids that are essential to growth and development. The mode of action of this family of proteins is well known (Alibhai and Stallings, 2001).

The shikimate pathway and EPSPS enzyme are found in plants and microorganisms but are absent in animals including mammals, fish, birds and reptiles (Alibhai and Stallings, 2001; Haslam, 1993). This explains the selective activity of glyphosate in plants and contributes to the low animal toxicity of glyphosate. Additionally the U.S. EPA has concluded that, when used according to label directions, glyphosate does not pose unreasonable risks or adverse effects to humans or the environment and has a favorable NTO safety profile (U.S. EPA, 1993a). APHIS also concluded in the final environmental impact statement for glyphosate tolerant alfalfa (USDA-APHIS, 2010) that glyphosate does not pose a risk to birds, mammals, fish, terrestrial invertebrates or aquatic invertebrates. APHIS further concluded that potential impacts to non-target plants may occur from drift resulting from the aerial application of glyphosate, but that these potential impacts have been mitigated through specific label use restrictions for glyphosate when applied with aerial equipment. Good management practices for application of all pesticides are also promoted by state university extension services (Jordan et al., 2009; University of Illinois, 2010). In addition, the majority (> 60% in 2008 (Zollinger et al., 2009) of U.S. canola acres are planted with glyphosate-tolerant canola and are routinely treated with glyphosate.

Taken together, these data support the conclusion that MON 88302 has no reasonable mechanism for harm to NTOs, or impact to threatened and endangered species compared to the cultivation of commercial canola. In addition, the U.S. EPA has taken measures (specified label language regarding glyphosate application) to minimize any potential effect to nontarget plants.

IX.C. Weediness Potential of MON 88302

Brassica napus is generally regarded as an opportunistic species, that is, a species adapted to take advantage of temporary conditions such as disturbed areas (CFIA, 1994), not as an environmentally hazardous colonizing (EC, 2000) or invasive species in undisturbed natural ecosystems (Crawley et al., 2001). Although *B. napus* has some characteristics typical of weedy species such as a high reproductive capacity, rapid growth and multiple pollination mechanisms (self, wind, insect), it also has many characteristics typical of domesticated species including low genetic diversity, lack of long-distance seed dispersal mechanisms, limited population persistence, lack of primary seed dormancy and an inability to compete well with perennial species (Hall et al., 2005). *Brassica napus* is not listed as an invasive weed in the Catalog of Invasive Plant Species of the U.S. (NYBG, 2011), nor is it present on the lists of noxious weed species maintained by the federal government (7 CFR § 360).

Brassica napus has been documented to be present in disturbed areas such as roadsides and railways used for transportation of seed and the margins of fields where it has been previously grown (Aono et al., 2006; Knispel et al., 2008; Nishizawa et al., 2009; Pivard et al., 2008; Saji et al., 2005). However, populations of canola outside agricultural fields do not effectively compete with perennial vegetation, and usually persist only for a few years in the absence of ongoing seed introductions into areas from spillage during handling and transport or processing (Baker and Preston, 2008; Crawley and Brown, 1995; 2004; Crawley et al., 1993; Knispel et al., 2008). The persistence of canola outside the agroecosystem has been studied extensively and is summarized in Table IX-1. Canola plants outside agricultural fields can produce seed (Crawley and Brown, 1995; Knispel et al., 2008) but this is often prevented because most plants do not survive to maturity. This is due to competition from other vegetation (Crawley and Brown, 1995), management operations such as roadside mowing, the use of broadleaf herbicides, animal predation, diseases and environmental conditions (Crawley and Brown, 1995; Knispel et al., 2008; Norris and Sweet, 2002; Yoshimura et al., 2006). Additionally, biotechnology-derived canola populations have not been found to be more invasive or more persistent than conventional canola populations (Crawley et al., 2001).

The viability of the large majority of canola seed in soil declines over time (Gulden et al., 2003; Gulden et al., 2004; Hails et al., 1997). Biotechnology-derived canola seed has not been demonstrated to persist longer than conventional canola seed (Gruber et al., 2004; Hails et al., 1997). However, canola seed can persist in the soil for several years becoming secondarily dormant, and then germinate as volunteers (Gruber et al., 2004). Canola that has germinated and emerged unintentionally in a subsequent crop, also known as volunteer canola, may compete with the succeeding rotational crop. However, problems controlling volunteer canola are not common (Boyles et al., 2009). Volunteers, including volunteers with herbicide-tolerant traits, can be managed with pre-plant or selective post-emergent herbicide applications or by mechanical means (Boyles et al., 2009; EC, 2000).

In comparative studies between MON 88302 and the conventional control, phenotypic, agronomic and environmental interaction data were evaluated (Section VII.C.2.) for

changes that would impact the plant pest potential, and, in particular, plant weediness potential. Results of these evaluations show that there is no biologically significant difference between MON 88302 and the conventional control for traits potentially associated with weediness such as germination, dormancy, reproductive growth and pollen characteristics, response to abiotic stress and disease or pest susceptibilities. Furthermore, comparative field observations between MON 88302 and the conventional control and their response to abiotic stressors, such as cold, drought, heat, nutrient deficiency, soil compaction, wet soil, and wind, indicated no differences and, therefore, no increased weediness potential. Collectively, these findings support the conclusion that MON 88302 has no increased weed potential compared to conventional canola and it is no more likely to become a weed than conventional canola.

Table IX-1. Summary of Literature on the Persistence of Canola Outside of Cultivated Fields

Reference	Methods	Results
(Nishizawa et al., 2009)	Three years of monitoring for canola on roadsides along routes for transportation of canola seed from the shipping port of Kashima (1 of 11 shipping ports for canola seed in Japan). Plants of *B. juncea* were also observed, and tested for the presence of glyphosate- and glufosinate-tolerance proteins and genes.	The number of plants found each year; along a 20 km section of roadway varied from 2162 plants in 2005, 4066 plants in 2006 and 278 plants in 2007. *Brassica napus* populations were found along roadsides or sidewalks, with some found in vacant lots and flower beds. Most plants found were *B. napus* but a few *B. juncea* plants were also observed. In all three years herbicide-tolerant canola plants were found, with the percentage of canola plants with either the glyphosate- or glufosinate-tolerance trait varying from 0 to 1.8%. The authors concluded that no massive expansion or long-term persistence of canola populations had occurred and that environmental impact from canola seed spillage along transportation routes would be negligible.
(Baker and Preston, 2008)	Monitoring of canola seed persistence in managed fields in Australia for up to 3.5 years after the crop was grown over 3 geographical regions from a total of 66 fields. Canola seeds were isolated and counted from soil samples taken from commercial fields.	The overall mean number of seeds recovered per meter squared that germinated was 3% for seed collected 6 months after harvest and for seed collected 1.5 and 2.5 years after the last canola crop, it was 1.4% and 1.5%, respectively. No seed collected 3.5 years after the last canola crop germinated. Seed viability was not tested in the study.
(Knispel et al., 2008)	Observations of canola plant densities along field edges and roadways in Manitoba Canada from 2004 to 2006. Sampling of seed from 16 canola populations and testing for herbicide tolerance. Soil sampling and testing for canola seed viability and dormancy.	Many canola plants along roadways died from mowing and most did not survive to set seed. Only 25% of the populations had plants that set seed over 2 years, and only one population set seed in all 3 years of the study. Herbicide-tolerant canola plants were detected at a high frequency in the populations sampled, and evidence of pollen-mediated gene flow of herbicide-tolerance traits was detected. Soil sampling from along roadways indicated that the seedbanks were small (less than 5 germinable seeds per square meter) and had little dormancy. The authors concluded that canola populations outside of cultivated areas are highly transient.

Table IX-1. Summary of Literature on the Persistence of Canola Outside of Cultivated Fields (continued)

Reference	Methods	Results
(Pivard et al., 2008)	Observations of canola along roadsides and field margins in France in a large-scale study over 4 years. Regression modeling was used to estimate the contribution of ecological processes involved in canola presence and persistence.	The presence of 35–40 % of the ruderal canola populations was associated with seed immigration from neighboring fields. Approximately 15 % of the populations were attributed to seed losses during transportation. The presence of no more than 10 % of the populations was attributed to seed produced by ruderal canola populations in the previous year. The remaining canola populations, 35–40 %, were attributed to recruitment from seed that had been in the seed bank for 1 or more years.
(Aono et al., 2006)	Screening of seed collected from canola (*B. napus*), *B. juncea* and *B. rapa* plants from 95 sites from 7 port areas in Japan for herbicide tolerance.	No biotechnology-derived seeds were found in *B. juncea* and *B. rapa* samples. Seeds were collected from canola plants and tolerance to glyphosate and glufosinate was found. A portion of the progeny of two individual plants had both the glyphosate-tolerance and glufosinate-tolerance traits.
(Yoshimura et al., 2006)	Monitoring of sites in Saskatchewan, where canola is grown, and in Vancouver, British Columbia, where most canola destined for export is transported by rail. Observations at both areas of railway and along side roads for *Brassica* species were conducted Plants were tested for glyphosate and glufosinate herbicide-tolerance proteins.	The glyphosate and glufosinate tolerance traits were found in approximately 2/3 of the canola plants sampled in Saskatchewan and British Columbia. In Saskatchewan and Vancouver plants of cultivated (163 samples) and weedy relatives of canola (223 samples) were screened for glyphosate and glufosinate tolerance traits. These relatives included wild mustard (*Sinapis arvensis*), oriental mustard (*B. juncea*), dog mustard (*Erucastrum gallicum*) and bird's rape (*B. rapa*). Among these samples only a single *B. rapa* × *B. napus* hybrid with the glyphosate-tolerance trait was detected in British Columbia.

Table IX-1. Summary of Literature on the Persistence of Canola Outside of Cultivated Fields (continued)

Reference	Methods	Results
(Lutman et al., 2005)	Comparison of seed losses by shattering at harvest and persistence in soil of glyphosate-, glufosinate- and imidazolinone-tolerant and conventional canola varieties at 5 sites over 4 years.	No difference between glyphosate-tolerant, glufosinate-tolerant, imidazolinone-tolerant, and conventional canola varieties for seed shattering at harvest and seed persistence in soil. The number of canola seeds of all varieties declined rapidly in the soil in the first few months after harvest with a mean loss of 60 %. The number of canola seeds in the soil declined by approximately 20 % per year for the remainder of the experiment.
(Saji et al., 2005)	Collection of seeds from canola, *B. rapa* and *B. juncea* found growing near ports, along roadsides and along riverbanks. Seeds were grown and tested with either of glufosinate or glyphosate herbicide, and survivors were tested for presence of both herbicide-tolerance proteins and genes.	Seeds were collected from 7500 feral *B. napus*, 300 *B. rapa* and 5800 *B. juncea* plants from 143 locations. No herbicide-tolerance genes or proteins were detected in *B. rapa* or *B. juncea* nor in *B. napus* plants found along riverbanks.
(Crawley and Brown, 2004)	Survey of *B. napus* populations in 100-m quadrants along roadside in the U.K. for 10 years (1993–2002)	Seed spillage from trucks was a significant contributor to the observed *B. napus* populations along the roadside. Plants were mostly observed within 1 m of the roadside and seldom in dense grass or in deep shade. In the years following road works *B. napus* population densities were significantly higher in 9 of 12 cases. Overall the populations were not self-replacing. Canola populations were extinct after 1 to 4 years. The most frequent pattern was for quadrants to have canola present for 1 to 2 years out of 10 with only 1 % of quadrants surveyed having one or more canola plants in all 10 years.
(Gruber et al., 2004)	Study of seed viability after burial in two separate years in the UK, with evaluation of seed viability of conventional and transgenic seeds after burial.	No differences between biotechnology-derived and the near isogenic counterparts were found for seed survival in soil. Mean canola seed viability of buried seed was 66 % and 33 % for the first and second years of the study, respectively.

Table IX-1. Summary of Literature on the Persistence of Canola Outside of Cultivated Fields (continued)

Reference	Methods	Results
(Gulden et al., 2004)	Laboratory and field-based studies of canola secondary dormancy and seed persistence in soil.	In the field experiments, canola viability after 4 weeks in soil ranged from 0.1% to 6.3%. Canola seedling recruitment from seed in the shallow soil seedbank was low, ranging from 0.1% to 1.5%. The authors conclude that germination may have contributed to loss of seed from the deep buried seedbank but because seedlings were not recruited from the deep seedbank depth (10 cm) germination at these deeper depths must have been lethal.
(Gulden et al., 2004)	Seeds were planted at two sites in Canada and persistence was measured each spring for 3 years.	Different canola varieties persisted for different lengths of time. After 1, 2 and 3 winters the maximum persistence was 44, 1.4 and 0.2 % of the original buried seeds, respectively, over all varieties. In 2001, canola genotypes with high potential for dormancy tended to exhibit 6- to 12-fold greater persistence than medium dormancy potential canola genotypes, indicating lower seedbank mortality in high potential dormancy varieties canola.
(Norris and Sweet, 2002)	Monitoring along field margins of a glufosinate-tolerant canola trial in the UK which had not previously been cropped to biotechnology-derived canola.	Only one persistent population of feral *B.* napus was found at any site in any year. The population persisted for 3 years. In the third year of monitoring all of the plants in the population were mowed late in the season, and no plants were found in the fourth year of monitoring. Feral populations of oilseed rape were rare in this study, and where populations were found in one year, these did not generally persist to following years. Individual plants growing in roadsides and verges rarely survived to maturity and set seed. Many factors were involved in the mortality of these plants, the main ones being competition with perennial weeds (especially grasses), predation by animals (slugs, birds and insects) and susceptibility to diseases of canola.
(Crawley et al., 2001)	Conventional and biotechnology-derived canola planted at 12 habitats and monitored for 10 years.	No difference in recruitment between conventional and biotechnology-derived canola. None of the conventional or biotechnology-derived canola increased in abundance. Biotechnology-derived canola did not persist longer than conventional canola. Establishment of seedlings was lower for biotechnology-derived canola than conventional at 6 of the 12 sites, and was not significantly greater at any site. Survival of biotechnology-derived canola relative to conventional canola was lower in 3 of 12 cases and higher in 2 of 12 cases. All populations were completely extinct after 4 years.

Table IX-1. Summary of Literature on the Persistence of Canola Outside of Cultivated Fields (continued)

Reference	Methods	Results
(Hails et al., 1997)	Seed burial study for 2 years at 12 four habitats (sites) in each of 3 different geographic sites in the UK (12 total habitats). The source of canola seed was the variety Westar for the conventional and biotechnology-derived lines (one antibiotic-tolerant, one herbicide-tolerant and a third with both traits). A wild relative, *Sinapis arvensis* (charlock or wild mustard), was also included.	After burial in soil only 1.5 % of the canola seeds persisted after the first year and 0.2% after the second year at all sites. The survival rate of biotechnology-derived seeds was lower than the conventional seeds at both burial depths at all 12 habitats. However, when individual habitats were examined this trend was only detectable at 5 habitats and no detectable difference at the other 7 habitats. The weed *S. arvensis* persisted significantly longer than any of the canola seeds in all habitats and sites.
(Crawley and Brown, 1995)	Monitoring for canola plants in road verges adjacent to the M25 highway (UK).	Canola plants were not seen in dense grass. Populations established by seed losses during transportation in the first year of the study were extinct within 4 years. In the absence of disturbance local canola populations were typically extinct within 2–4 years. In the year following soil disturbance canola densities were often high.
(Crawley et al., 1993)	Experiment to examine whether biotechnology-derived herbicide-tolerance affects the likelihood of *B. napus* becoming invasive of natural habitats.	No evidence of invasiveness of canola in undisturbed natural habitats. No difference in invasiveness or persistence between biotechnology-derived and conventional varieties. Local canola populations measured were declining and local extinction in all habitats was likely. The authors concluded that there is no evidence that *B. napus* is invasive of undisturbed natural habitats.

IX.D. Potential for Pollen Mediated Gene Flow and Introgression

Pollen mediated gene flow (often referred to as cross pollination) occurs when pollen of one plant fertilizes ovules of a second plant. Pollen mediated gene flow is affected by both biotic and abiotic factors such as plant biology, pollen biology/volume, plant phenology, overlap of flowering times, proximity of the pollen source and sink, ambient conditions such as temperature and humidity and field architecture. Because pollen mediated gene flow is a natural biological process, it does not constitute an environmental risk in and of itself.

Introgression is a process whereby one or more genes successfully incorporate into the genome of a recipient plant. Pollen mediated gene flow and gene introgression must be considered in the context of the transgenes inserted into the biotechnology-derived plant, and the likelihood that the presence of the transgenes and their subsequent transfer to recipient plants and plant populations will result in increased plant pest potential. The potential for gene flow and introgression from deregulation of MON 88302 is discussed in greater detail below.

IX.D.1. Potential for Pollen Mediated Gene Flow and Introgression of *B. napus*

Brassica napus is predominantly self-pollinating although interplant (plants are touching one another) cross pollination rates range from 12% to 55% with a mean of 30% (Beckie et al., 2003). Pollen of *B. napus* is heavy and sticky (OECD, 1997) and pollen movement is primarily by insects, such as honey bees (Thompson et al., 1999) although wind is also responsible for some pollen movement. Most (98.8%) of pollen travels less than twelve meters from its source (Scheffler et al., 1993) although dispersal due to pollinators may occur over greater distances at low frequency (Thompson et al., 1999). In general, the percentage of pollen flow and potential for cross pollination diminishes with increasing distance from the source (Table IX-2). This information is useful for managing pollen flow during canola breeding, seed production and for identity preservation.

Brassica napus produces a large amount of pollen (OGTR, 2008) which can remain viable up to four to five days under field conditions (Rantio-Lehtimäki, 1995). This, coupled with the potential for *B. napus* pollen movement, suggests the possibility for hybridization between *B. napus* and related species.

Table IX-2. Summary of Representative Studies of *Brassica napus* Cross Pollination Rates at Various Distances

Reference	Distance from Pollen Source (meters)	% Cross Pollination Observed
(Stringam and Downey, 1982)	47	2.1
	137	1.1
	366	0.6
(Manasse and Kareiva, 1991)	50	0.022
	100	0.011
(Scheffler et al., 1993)	0	4.8
	1	1.4 – 1.6
	3	0.35 – 0.4
	6	0.033 – 0.11
	12	0.016 – 0.025
	24	0.0 – 0.0041
	36	0.0011 – 0.0031
	47	0.0 – 0.00034
	70	0.0
(Morris et al., 1994)	0	~2.0 – 3.5[§]
	0.3	~1.0 – 1.5[§]
	0.6	~0.75 – 1.2[§]
	3	~0.5 – 0.75[§]
	4.6	~0.5 – 0.7[§]
(Scheffler et al., 1995)	200	0.0156
	400	0.0038
(Downey, 1999)	33	0.1 – 1.5
	66	0.0 – 0.4
	100	0.1 – 0.4
(Staniland et al., 2000)	0	0.69
	2.5	0.29
	5	0.14[φ]
	10	0.07[φ]
	15	0.08[φ]
	20	0.07[φ]
	25	0.04[φ]
	30	0.02[φ]
(Rieger et al., 2002)	100	0.012 – 0.014
	1500	0 – 0.197
	>3000	None detected

[§]Frequencies estimated from Figure within the publication.
[φ]Values calculated from publication.

IX.D.2. Potential for Pollen Mediated Gene Flow and Introgression of *B. napus* to Closely Related *Brassica* Species

A survey of the literature provides information on the potential for hybridization (cross pollination) and introgression from *B. napus* to related species. Reports of unassisted (without human intervention or assistance) field hybridization of *B. napus* to related species provide biologically relevant information that can be useful in predicting the potential for hybridization under field conditions. However, the majority of the published information used to assess hybridization of *B. napus* with related species is from the breeding literature where human assistance has occurred. Often these studies involve emasculation or male-sterility of the female parent, assisted pollination by hand or the use of cages and high numbers of pollinators (*e.g.,* bees). In addition, cross-species hybrids have also been obtained using *in vitro* methods like embryo or ovary culture. Although, methods using assistance or *in vitro* techniques may not be indicative of the possibility of hybridization potential under field conditions, they do provide information about hybrid viability, fertility, chromosome behavior and the potential for introgression into a *B. napus* relative, if hybrids were to form.

The frequency of hybridization between *B. napus* and several of its relatives is often more successful when *B. napus* is the male parent in some crosses or the female parent in others. This directional bias has been observed in a number of studies (Scheffler and Dale, 1994). In the case of MON 88302, transfer of characteristics from other species to *B. napus* is not a primary concern in evaluating environmental impacts since it is highly unlikely that transfer of characteristics will result in an increase in the weediness of *B. napus*. If such a transfer were to occur, the resulting hybrids will likely be present in cultivated fields and subject to the same agricultural management practices as existing herbicide-tolerant canola varieties that would control their ability to grow and reproduce. Thus, for the purposes of MON 88302 risk assessment, hybridizations in which *B. napus* is the male parent were carefully reviewed. There are reports of hybridization under field conditions with *B. napus* as the pollen donor with six species including *B. rapa*, *B. juncea*, *B. oleracea*, *H. incana*, *R. raphanistrum* and *S. arvensis* (Table IX-3). The species *B. rapa*, *B. juncea* and *B. oleracea* are cultivated for crop production. The other species listed, *H. incana*, *R. raphanistrum* and *S. arvensis* are not cultivated for crop production, but are found in the environment. In all cases the resulting hybrids had decreased environmental fitness evidenced by a variety of characteristics including decreased pollen viability, seed production, seedling survival, *etc*. when compared to parental varieties.

Brassica rapa

Brassica rapa is widespread throughout temperate North America, and may be found in areas where canola is grown (BONAP, 2010a). *Brassica napus* (AACC) was derived from the hybridization of diploid species, *B. rapa* (AA) and *B. oleracea* (CC) (Section II). Therefore *B. napus* and *B. rapa* have a common set of chromosomes, are known to be sexually compatible, and can form hybrids under field conditions (Bing et al., 1991; Warwick et al., 2003). For pollination, *B. rapa* is self-incompatible, and is not likely to form hybrids when in close proximity to con-specifics (members of the same species)

(Warwick et al., 2003). Hybridization frequencies between *B. napus* and *B. rapa* in neighboring fields or inter-planted in large plots vary from study to study, ranging from 0% up to 69% (as reviewed in (Devos et al., 2009), but can be as high as 93% (Jørgensen et al., 1996). For example, Warwick (2003) measured hybridization frequencies between these two species with *B. rapa* as the maternal parent in field experiments, including natural environments and commercial *B. napus* fields. The higher rate of hybridization in commercial fields (13.6%) vs. natural environments (7%) is due to the higher pressure of *B. napus* pollen on isolated plants of self-incompatible species, like *B. rapa*, favoring inter-specific hybridization. Further evidence for this were the lower hybridization frequencies (0.4% to 1.5%) when *B. napus* was the male parent with *B. rapa* female parent plants located outside of cultivated fields of *B. napus* (Scott and Wilkinson, 1998). F_1 hybrids between *B. napus* and *B. rapa* had reduced pollen viability with an average viability of approximately 50% (Warwick et al., 2003). F_1 hybrids between *B. napus* and *B. rapa* produced approximately half as many germinable seeds as their respective parents, and had very low (< 2%) hybrid seedling survival rates (Scott and Wilkinson, 1998). Additionally, unlike the *B. rapa* parent, under field conditions, seeds of F_1 hybrids between these two species lacked seed dormancy (Jørgensen et al., 1998) which may limit F_1 hybrid persistence. In a field study backcrosses with *B. rapa* as the female parent with F_1 and BC_1 hybrids occurred at very low frequencies with an overall mean of 0.088% (Halfhill et al., 2004).

The presence of a herbicide-tolerance trait (glufosinate) introgressed experimentally under controlled conditions from *B. napus* into *B. rapa* did not increase its survival or number of seeds per plant compared to parental *B. rapa* (Snow et al., 1999). In fact, F_1 hybrids, F_2 plants, and backcross generations with *B. rapa* as the female parent, had fewer pods and viable seeds than those of the *B. rapa* parent due in part to reduced vegetative vigor and photosynthetic capability (Ammitzboll et al., 2005; Hauser et al., 2003; Hauser et al., 1998). F_1 hybrids between *B. napus* and wild *B. rapa* demonstrated a lower vegetative performance (seedling height and seedling biomass) than the parents when compromised by selection pressures simulated herbivory and interspecific competition (Sutherland et al., 2006).

Introgression, *i.e.*, the stable incorporation of genes from one differentiated gene pool into another, of a biotechnology-derived trait from *B. napus* to *B. rapa* has been demonstrated on a single occasion under commercial field conditions (Warwick et al., 2008). Populations of *B. rapa* located close to commercial fields of glyphosate-tolerant *B. napus* were monitored for multiple years. The number of hybrids with the glyphosate-tolerance trait declined drastically from the first to fifth year of monitoring, but persisted at low levels at one of the two sites. A single glyphosate-tolerant diploid individual with 29% pollen viability was discovered five years after the last planting of glyphosate-tolerant *B. napus* confirming the presence of the herbicide tolerance trait over time (Warwick et al., 2008).

These data support the conclusion that hybridization between *B. napus* and *B. rapa* is possible under field conditions, but resulting progeny have decreased fitness. In the rare occurrence that the glyphosate-tolerance trait introgressed and persistence of hybrids between MON 88302 and *B. rapa* were to occur, these plants could be controlled by

mechanical or chemical means (Section VIII.H.). Additionally, in Canada, with twelve years of experience growing millions of acres of herbicide-tolerant canola each year, no significant agronomic problems with herbicide tolerant *B. rapa* have been encountered (Beckie, 2006).

<u>*Brassica juncea*</u>

Brassica juncea is sparsely but widely distributed in temperate North America in cultivated and disturbed areas, and may be found in areas where canola is grown (BONAP, 2010a). *Brassica juncea* is an amphidiploid (AABB) derived from the hybridization of *B. nigra* (BB) and *B. rapa* (AA) (Section II). *Brassica napus* and *B. juncea* have a common set of chromosomes (AA), are known to be sexually compatible, and can form hybrids under open pollination conditions (Bing et al., 1996; Frello et al., 1995; Jørgensen et al., 1998). *Brassica juncea* is a predominantly self-pollinating species with approximately one third of pollination due to insect pollination (Duke, 1997). As reviewed in Devos (2009), in field plots with interplanted *B. napus* and *B. juncea* interspecific hybridization frequencies were low ranging from 0.3% to 3%. F_1 hybrids between *B. juncea* and *B. napus* had reduced male and female fertility, very low pollen viability ranging from 0% to 28% and low seed set (Bing et al., 1996; Frello et al., 1995). The majority of studies of hybridization between *B. napus* and *B. juncea* have shown some directional effects such that F_1 hybrid seed production is much lower in some studies when *B. napus* is the female parent, but lower in other studies when *B. juncea* is the female parent. Bing et al., (1996) conducted field experiments with neighboring *B. juncea* and *B. napus* plants to assess the potential for hybridization under field conditions. When *B. napus* was the female, the authors found five hybrid seeds from 469 plants (1.1%); when *B. juncea* was the female, they found three hybrid seeds from 990 plants (0.3%). Hybrid seeds formed when *B. napus* was the female parent were often deformed (*e.g.,* without an embryo) or they started to germinate in the seed pod (Frello et al., 1995). Thus, *B. napus* and *B. juncea* can hybridize at a low rate under field conditions, but the resulting hybrids have reduced male and female fertility significantly decreasing the likelihood of introgression.

<u>*Brassica oleracea*</u>

Brassica oleracea is distributed primarily in northeastern and midwestern states, and may be found in areas where canola is grown (BONAP, 2010a). The CC genome of *B. napus* was derived from *B. oleracea* (Section II). Therefore it is not surprising that *B. napus* and *B. oleracea* are known to have some limited sexual compatibility under open pollination conditions (Ford et al., 2006). *Brassica oleracea* is predominately a self-pollinating species (Devos et al., 2009) limiting its potential for hybridization with *B. napus*. In field plots with inter-planted *B. napus* and *B. oleracea*, formation of F_1 hybrid seed was reported when *B. napus* was the male parent, but the viability of the seed was not assessed (Chevre et al., 1998). In a field survey of co-localized *B. napus* and *B. oleracea* populations no hybrids with *B. napus* as the female parent were reported (Wilkinson et al., 2000). At a site in the UK where *B. napus* grew near wild *B. oleracea,* one triploid hybrid (0.1%) was detected out of 842 seed samples from two sites (Ford et

al., 2006). Thus, hybridization between these two species under field conditions appears to be relatively rare and introgression under field conditions is unlikely to occur.

Brassica nigra

Brassica nigra is widespread in temperate North America, and may be found in areas where canola is grown (BONAP, 2010a). No hybrids were produced in field plots with inter-planted *B. napus* and *B. nigra* (Bing et al., 1996). Even under controlled conditions, hybrids between these two species were not obtained using hand pollination, only when embryo rescue was performed and only when *B. napus* was the female parent. Reduced pollen fertility (0-1.9%) in the resulting hybrids (Kerlan et al., 1992) ensures that even if such a cross were to occur, reduced reproductive success makes introgression highly unlikely. Further, no hybrid plants were obtained when *B. napus* was the male parent (Kerlan et al., 1992). Therefore, the likelihood of gene flow from *B. napus* to *B. nigra* under field conditions is extremely low.

Brassica carinata

No field studies of the hybridization potential between *B. napus* and *B. carinata* have been reported (FitzJohn et al., 2007). Manual pollination results in only 0.08 seeds produced per pollination (Getinet et al., 1997) making gene flow under field conditions highly unlikely.

IX.D.3. Potential for Pollen Mediated Gene Flow and Introgression of *B. napus* to *Brassica* Vegetables

Gene flow from *B. napus* canola to *B. napus* vegetables (*e.g.,* Swedes or rutabaga, Siberian kale) is possible as they are members of the same species. As previously described, since *B. napus* has chromosomes in common with *B. rapa* and *B. oleracea*, *B. napus*, gene flow to *B. rapa* vegetables (*e.g.,* turnip and Chinese cabbage) and *B. oleracea* vegetables (*e.g.,* cabbage, cauliflower, broccoli, collards, kale, Brussels sprouts) is less likely but may occur. However, *B. napus*, *B. rapa* and *B. oleracea* vegetables are not considered weedy, and are generally harvested prior to flowering, preventing cross-pollination, hybridization and seed formation. Thus the potential for *B. napus* gene flow and introgression into closely related vegetable species is low. Co-existence of cultivated *B.* napus with closely related vegetable species is discussed in greater detail in Appendix I..

IX.D.4. Potential for Pollen Mediated Gene Flow and Introgression of *B. napus* to Related Species in the Family Brassicaceae

Other species in the family Brassicaceae with which *B.* napus is sexually compatible under field conditions includes *Hirschfeldia incana, Raphanus raphanistrum* and *Sinapis arvensis*. A survey of the literature provides information on the potential for hybridization (cross pollination) and introgression from *B. napus* to these species.

Hirschfeldia incana

Hirschfeldia incana is found primarily along roadsides, in ditches and waste areas in California, Arizona, Oregon and Nevada (BONAP, 2010b). With the exception of Oregon, canola is not usually grown in these states. In Oregon in 2010 approximately 6000 acres, or less than 1% of total U.S. canola acres, were planted (USDA-NASS, 2011a).

In a field study with interplanted *B. napus* and *H. incana* plants the mean hybridization frequency with the *H. incana* as the female parent was 1.9 % (16 hybrids from a total of 853 seedlings) (Lefol et al., 1996b). The fitness of the F_1 hybrids produced was approximately 10^{-6} relative to the *H. incana* parent (Lefol et al., 1996b). In a two year field study evaluating the potential for introgression of a herbicide-tolerance gene from *B. napus* to *H. incana*, from 0.17% to 0.79% hybrid seed was produced (Darmency and Fleury, 2000). F_1 hybrid plants obtained from both the field experiment and from a hand pollination experiment in a greenhouse were backcrossed to *H. incana*. The herbicide-tolerant progeny in each generation were selected for further backcrossing. In each subsequent backcross generation, fewer seeds were produced and by the fifth backcross generation no viable seed were produced (Darmency and Fleury, 2000). The chromosome numbers of the plants in each backcross generation were irregular (Darmency and Fleury, 2000), and it has been suggested that genetic factors in *H. incana* limit homoeologous pairing, resulting in an elimination of *B. napus* chromosomes (Eber et al., 1994). In a subsequent evaluation of the F_1 hybrid seed produced from field studies, the survival and emergence after burial in soil under field conditions was assessed. Prior to burial in soil the F_1 hybrid seed had 97% germination, similar to the *B. napus* parent. However, hybrid plants behaved similarly to *B. napus*, and seedling emergence decreased to 1% after three years (Chadoeuf et al., 1998). Thus, although F_1 hybrids may occur with *B. napus* in areas where *H. incana* is found, introgression of *B. napus* genes into *H. incana* is not likely to be a significant phenomenon (Darmency and Fleury, 2000).

Raphanus raphanistrum

Raphanus raphanistrum is widely distributed in North America with the exception of the central Great Plains and may be found where canola is grown (BONAP, 2010c). In field experiments where both *B. napus* and *R. raphanistrum* were planted, interspecific hybrids were detected at very low frequencies in France at 0.001-0.00001% (Chevre et al., 2004), Australia at 0.000004% (Rieger et al., 2001), and Canada at 0.003% (Warwick et al., 2003). However, in surveys of large experimental fields where herbicide-tolerant *B. napus* and *R. raphanistrum* were both present, no hybrids between *B. napus* and *R. raphanistrum* were detected over five years in the UK, and over two years in Canada (Warwick et al., 2003). The frequency of crossing between *B. napus* canola and *R. raphanistrum* is extremely low especially with *R. raphanistrum* as the female parent (Gueritaine et al., 2002). As reviewed in Devos et al. (2009), seed dormancy of hybrids of *B. napus* and *R. raphanistrum* was within the range of their original parents and the hybrid plants had delayed seedling emergence, lower survival compared to both parents and produced less than two seeds per plant. Hybrids between these two species have a reduced pollen viability of less than 1% (Warwick et al., 2003).

Studies of inheritance of *B. napus* genes in hybrids between herbicide-tolerant *B. napus* and *R. raphanistrum* over four backcross generations to *R. raphanistrum* demonstrated that each successive generation had a reduction in chromosome number. By the fourth backcross generation, the hybrids had a chromosome number close to *R. raphanistrum* and the percentage of herbicide tolerant plants also decreased (Chevre et al., 1997). In another study (Gueritaine et al., 2002) DNA content and seed production were measured in sixth generation hybrids of *B. napus* and *R. raphanistrum*. Herbicide-tolerant hybrids were found to have higher DNA content and produce half the number of seeds than non-herbicide-tolerant plants and the *R. raphanistrum* control.

The potential for hybridization between *B. napus* and *R. raphanistrum* under field conditions is extremely low, and, if it were to occur, the hybrids would have reduced reproductive success. Therefore, introgression of a herbicide tolerance gene is very unlikely.

Sinapis arvensis

Sinapis arvensis is found abundantly in temperate agricultural areas of North America, and may be found in areas where canola is grown (BONAP, 2010d). Hybrids between *B. napus* and *S. arvensis* were not found in the field except under controlled conditions (Bing et al., 1996; Chèvre et al., 1996; Moyes et al., 2002). Hybrids in which *S. arvensis* is the female parent have not been reported without the use of *in vitro* techniques (Chèvre et al., 1996; Moyes et al., 2002). Hybrids developed using hand pollination demonstrated poor pollen viability and fertility and were unable to backcross with *S. arvensis* (Moyes et al., 2002).

In one report a single hybrid plant was found along the margins of a field trial containing biotechnology-derived herbicide-tolerant *B. napus* canola (Daniels et al., 2005). It demonstrated herbicide tolerance and the presence of the gene was confirmed using PCR techniques. In the following growing season seeds were harvested from the *S. arvensis* population at this same location, but none were found to be tolerant to the herbicide (Daniels et al., 2005). Thus, hybridization between *B. napus* and *S. arvensis* occurs at an extremely low frequency making introgression very unlikely under field conditions.

Eruca vesicaria

Eruca vesicaria is distributed throughout North America with the exception of southeastern U.S. and may be found in areas where canola is grown (USDA-NRCS, 2010a). No field studies on the hybridization potential between *B. napus* and *E. vesicaria* have been reported. Using protoplast fusion, hybrids can be produced (Fahleson et al., 1988). F_1 hybrids have limited pollen fertility and seed set relative to their parents. Therefore the likelihood of introgression of genes from *B. napus* into *E. vesicaria* is virtually non-existent and has not been reported in the literature.

Other Species in the Family Brassicaceae

Under field conditions hybrids have not been reported between *B. napus* and *Diplotaxus muralis*, *Diplotaxus tenuifolia* (Salisbury, 2002), *Erucastrum gallicum* (Warwick et al.,

2003), *R. sativus* or *S. alba* (Daniels et al., 2005; Norris and Sweet, 2002). Cross pollination of *B. napus* with any of these species is highly unlikely.

Table IX-3. Summary of Published Literature on Unassisted Hybridization under Field Conditions with *B. napus* as the Male Parent

Species	F_1 Hybrids	F_2 or Backcross (BC) Hybrids	Comments	Reference
B. rapa	160 hybrids	N/A	*Brassica napus* and *B. rapa* cross readily when either is the male or female parent. Self-sterile *B. rapa* produced many hybrid progeny when compatible pollen from other *B. rapa* plants was not available and few hybrids when *B. rapa* pollen was available.	(Palmer, 1962)
B. rapa	18 putative F_1 hybrids.	All F_1s were self-sterile but backcrosses with both parents yielded some seed.	Seed formed on recipients from the crosses was planted and the progeny visually assessed for morphological variants indicative of putative hybrids.	(Leckie et al., 1993)
B. rapa	Some populations did not have any hybrids. In one population, flowering near a winter *B. napus* crop, identified 33 hybrids among the 952 plants examined.	N/A	*B. rapa* plants with well developed seed pods were collected from six Danish localities and planted in greenhouse. Seeds were harvested from each individual plant at maturity. Potential hybrids with *B. napus* were selected based on morphology and a subset characterized with isozymes.	(Landbo et al., 1996)
B. rapa	9% to 93% depending on experimental design.	N/A but pollen fertility of the F_1 hybrids under field conditions ranged form 12 to 54% (pollen fertility of the *B. rapa* and *B. napus* parents were 89%-99%, and 99%-100%, respectively)	When *B. rapa* was the female parent F_1 seed germination was 55% with a seed dormancy breaking treatment. The frequency of hybrids when *B. rapa* was planted at low density (93%) among *B. napus* plants was much higher than when the two were planted in 1:1 ratio (13%).	(Jørgensen et al., 1996)
B. rapa	More than 4000 seeds from 32 hybrids.	F_1 hybrids crossed with *B. rapa* had pronounced seed dormancy. BC_1 crossed with *B. rapa* resulted in an average 6.4 viable BC_2 offspring plants per pollination.	From 44 hybrids selected with *B. rapa*-like morphology some had 20 chromosomes (like *B. rapa*) and greater than 90% pollen fertility.	(Mikkelsen et al., 1996)

Table IX-3. Summary of Published Literature on Unassisted Hybridization under Field Conditions with *B. napus* as the Male Parent (continued)

Species	F_1 Hybrids	F_2 or Backcross (BC) Hybrids	Comments	Reference
B. rapa	0.09% and 1.5% F_1 hybrid seed of the total harvested seed.	BC_1 failed under field conditions when *B. rapa* was the female.	Field trials produced F_1 seed. *B. rapa* was sown in the field with interspecific F1 hybrids, but backcrosses between the hybrid and *B. rapa* as the female were not observed. Under field conditions, backcrosses with *B. rapa* as the female parent were attempted but did not produce seed. F_1 hybrids produced approximately 2.5 seeds per pod, whereas, the original parents had 16 to 23. The seeds of the F_1 hybrids showed almost no sign of dormancy, which the authors suggest could limit interspecific gene flow because germination under unfavorable conditions (*e.g.,* with effective weed management) may occur.	(Jørgensen et al., 1998)
B. rapa	1 F_1 hybrid from 505 (0.2%) plants screened from sympatric locations.	N/A	Remote sensing was used to find potential areas of sympatry between *B. napus* and populations of *B. rapa* in England. Two *B. rapa* populations sympatric with *B. napus* were found. No *B. rapa* plants were observed in any cultivated fields in the survey, indicating there is negligible scope for transgene movement into natural *B. rapa* from *B. napus* x *B. rapa* hybrids formed in agricultural fields.	(Wilkinson et al., 2000)
B. rapa	F_1 hybrid frequency with different *B. napus* lines ranged from 0.7% to 16.9%.	Backcross seed could only be produced with half of the *B. napus* lines in the greenhouse.	Field experiments with 1200:1 ratio of *B. napus* to *B. rapa*.	(Halfhill et al., 2002)
B. rapa	F_1 hybrid frequency was 7% and 13.6% in field experiments and commercial fields, respectively.	F_1 hybrids had ~54% (range of two populations was 20-77% and 10-86%) pollen viability.	From field experiments a total of 32,154 seedlings were screened and from the commercial fields 9567 seedlings were screened.	(Warwick et al., 2003)

Table IX-3. Summary of Published Literature on Unassisted Hybridization under Field Conditions with *B. napus* as the Male Parent (continued)

Species	F_1 Hybrids	F_2 or Backcross (BC) Hybrids	Comments	Reference
B. rapa	F_1 hybrid frequency with different *B. napus* lines ranged from 4% to 22% with an overall mean of 10%.	When *B. rapa* was the female parent backcross frequencies from two locations were 0.088% and 0.06%.	A 600:1 ratio of *B. napus* to *B. rapa* with several different transformed lines of the former. Screened 12,388 *B. rapa* seedlings.	(Halfhill et al., 2004)
B. juncea	Mean F_1 hybrid frequency of 3% under field conditions.	Two F_1 hybrids with ~24% and 28% pollen fertility were used in backcrosses to *B. juncea*. The backcrosses in the greenhouse produced 148 seeds with 75% germination compared to 90-100% for the initial cross.	F_1 hybrid seed germination was 93% and pollen fertility ranged from 0% to 31%. BC_1 pollen fertility ranges from 24% to 90%, but note the study only used the highest pollen fertility F_1 hybrids for these crosses.	(Frello et al., 1995)
B. juncea	3 F_1 hybrids from 990 *B. juncea* plants as the female parent.	N/A	Two experiments consisting of (1) five-row plots with 61 cm inter-row spacing with a 50:50 mixture of the co-cultivating species sown in the center row, or (2) four-row plots with 25 cm inter-row spacing, 6 m long, with female plants transplanted at low density inter-row.	(Bing et al., 1996)
B. juncea	2.3%, 1.1% and 0.3% hybrids in co-cultivation plots with 1:3, 1:10 and 1:15 ratio of *B. napus* to *B. juncea* plants, respectively.	BC_1 with *B. juncea* as the female parent.	Results from backcrosses reported in Frello (1995).	(Jørgensen et al., 1998)
B. oleracea	0 F_1 hybrids from one *B. oleracea* population sympatric with a *B. napus* field.	N/A	Remote sensing was used to identify potential sites of sympatry between *B. napus* production fields and populations of *B. oleracea* in England. Every newly recruited plant in the population was screened for hybrid status using flow cytometry and molecular analyses.	(Wilkinson et al., 2000)

Table IX-3. Summary of Published Literature on Unassisted Hybridization under Field Conditions with *B. napus* as the Male Parent (continued)

Species	F_1 Hybrids	F_2 or Backcross (BC) Hybrids	Comments	Reference
B. oleracea	1 triploid F_1 hybrid,.	Twelve tentative introgessants were identified with *B. napus* specific markers from 842 (1.4%). Of these nine were diploid and two near triploid hybrids	A single F1 hybrid from 842 samples did not provide sufficient statistical power to provide an accurate estimate of hybridization frequency. The 12 tentative introgressed genotypes with crop-specific markers (3 triploids and nine diploids) out of 842 provided a frequency of 1.4%.	(Ford et al., 2006)
B. nigra	0 F_1 hybrids from 198 *B. nigra* plants.	N/A	Male parents were sown in four-row plots, 6 m long, with female plants transplanted at low density inter-row.	(Bing et al., 1996)
B. nigra	No hybrids.	N/A	No hybrids were observed form *B. nigra* plants surrounded by *B. napus* plants in cages with blowflies as pollinators	(Leckie et al., 1993)
H. incana	0 F_1 in experiment with cages from 36, 900 seeds screened and 16 F1 hybrids from 1061 in open field (~1/plant)	N/A	An experiment with cages and bees with *B. napus* and *H. incana* plants produced no hybrids. F_1 hybrids produced almost no pollen and the pollen grains were aborted. On average F_1 hybrids produced 40% fewer flowers, 20x fewer pods/flower, 50x fewer seeds/pod and $2x10^5$ fewer seeds/plant than *H. incana*. Of the 168 F_1 hybrids only 32 seeds were collected of which only 5 germinated. The seeds that did germinate 3 were completely sterile, 1 produced 5 viable seeds and 1 produced 57 viable seeds.	(Lefol et al., 1996b)
H. incana	Mean of 0.6 F_1 hybrid seeds per plant.	Backcrossing interspecific hybrids to *H. incana* over five generations showed that introgression was not successful.	Seeds output in each backcross generation decreased and no viable seeds were produced by the 5^{th} generation.	(Darmency and Fleury, 2000)

Table IX-3. Summary of Published Literature on Unassisted Hybridization under Field Conditions with *B. napus* as the Male Parent (continued)

Species	F_1 Hybrids	F_2 or Backcross (BC) Hybrids	Comments	Reference
R. raphanistrum	2 F_1 seeds from 147,671 total seeds.	Seed production of F_1 hybrids and their F_2 descendants was up to 0.4% and 2%, respectively, of that of *R. raphanistrum*.	Male-fertile *B. napus* in cages with different proportions of *R. raphanistrum* and bees did not produce hybrids. Two hybrids produced under field conditions without cages.	(Darmency et al., 1998)
R. raphanistrum	1 F_1 hybrid from 189,084 seedlings obtained from seeds harvested from *R. raphanistrum* plants.	N/A	The 1 F_1 hybrid obtained in the study had pollen fertility of 6.5%.	(Chevre et al., 2000)
R. raphanistrum	0 F_1 hybrids from 25,000 seeds harvested from *R. raphanistrum* plants.	N/A	Two hybrids were obtained when *B. napus* was the female parent from 52-million seeds screened (frequency of 4 x 10^{-8}).	(Rieger et al., 2001)
R. raphanistrum	1 F_1 hybrid from 32,821 seedlings screened.	N/A	F_1 hybrid had <1% pollen viability.	(Warwick et al., 2003)
R. raphanistrum	0 F_1 hybrids from 19,274 seeds harvested.	N/A	Ratio of 6001 of *B. napus* to *R. raphanistrum*.	(Halfhill et al., 2004)
S. arvensis	1 putative F_1 hybrid.	N/A	Blowflies were added to cages to encourage pollen movement. The chromosome number and fertility of the single hybrid was not confirmed in this publication.	(Leckie et al., 1993)
S. arvensis	0 F_1 hybrids from 61 *S. arvensis* plants.	N/A	Male parents were sown in four-row plots, 6 m long, with female plants transplanted at low density inter-row.	(Bing et al., 1996)
S. arvensis	0 F_1 hybrid seeds from 2.9 million seeds harvested.		Artificial hybrids grown in the presence of *S. arvensis*, or hand-crossed, produced a few aborted seeds..	(Lefol et al., 1996a)
S. arvensis	1 F_1 hybrid.	The F_1 hybrid did not have viable pollen. The F_1 hybrid produced no seed.	The authors concluded that the risk of gene transfer from *B. napus* to *S. arvensis* is minimal.	(Moyes et al., 2002)
S. arvensis	0 F_1 hybrids.	N/A	Screened approximately 43,000 seedlings of *S. arvensis* and no hybrids were detected.	(Warwick et al., 2003)

IX.D.5. Transfer of Genetic Information to Species with Which *Brassica napus* Cannot Interbreed (Horizontal Gene Flow)

Monsanto is not aware of any reports confirming the transfer of genetic material from *B. napus* to other species with which *B. napus* cannot sexually interbreed. The probability for horizontal gene flow to occur is judged to be exceedingly small. Even if it were to occur, the consequences would be no greater than from any other herbicide-tolerant canola currently available. Furthermore, MON 88302 produces the identical CP4 EPSPS protein as is found in commercial Roundup Ready crop products including Roundup Ready canola (RT73) and has been shown to have no meaningful toxicity to humans and to other NTOs under the conditions of use.

IX.E. Potential Impact on Canola Agronomic Practices

An assessment of current canola agronomic practices was conducted to determine whether the cultivation of MON 88302 has the potential to impact current canola and weed management practices (Section VIII). Canola fields are typically managed agricultural areas that are dedicated to crop production. MON 88302 is likely to be used in common rotations on land previously used for agricultural purposes. Certified seed production will continue to use well-established industry practices to deliver high quality seed containing MON 88302 to growers. Cultivation of MON 88302 is not expected to differ from current canola cultivation using glyphosate-tolerant canola, with the exception of an opportunity to use glyphosate during an expanded period of application and at rates higher than those currently recommended and authorized.

MON 88302 is similar to conventional canola in its agronomic, phenotypic, ecological and compositional characteristics and has levels of resistance to insects and diseases comparable to conventional canola. Therefore, no significant impacts on current cultivation and management practices for canola are expected following the introduction of MON 88302. Based on this assessment, the introduction of MON 88302 will not impact current U.S. canola cultivation practices or weed management practices, other than intended weed control benefits.

IX.F. Summary of Plant Pest Assessments

Plant pests, as defined in the Plant Protection Act, are living organisms that can directly or indirectly injure, cause damage to or cause disease to any plant or plant product (7 U.S.C. § 7702[14]). Data presented in Sections V through VII of this petition confirm that MON 88302, with the exception of glyphosate tolerance, is not significantly different from conventional canola, in terms of pest potential. Monsanto is not aware of any study results or observations associated with MON 88302 that would suggest that an increased plant pest risk would result from its introduction.

The plant pest assessment was based on multiple lines of evidence developed from a detailed characterization of MON 88302 compared to conventional canola, followed by a risk assessment on detected differences. The plant pest risk assessment in this petition was based on the following lines of evidence: 1) insertion of a single functional copy of

the *cp4 epsps* expression cassette; 2) characterization of the CP4 EPSPS protein expressed in MON 88302; 3) safety of the CP4 EPSPS protein; 4) compositional equivalence of MON 88302 seed as compared to a conventional control; 5) phenotypic and agronomic characteristics demonstrating no increased plant pest potential; 6) negligible risk to NTO and threatened or endangered species; 7) familiarity with canola as a cultivated crop and 8) no greater likelihood to impact agronomic practices, including land use, cultivation practices, or the management of weeds, diseases and insects, than conventional canola.

Based on the data and information presented in this petition, it is concluded that, similar to the currently deregulated canola products, MON 88302 is highly unlikely to be a plant pest. Thus, the results support a conclusion of no increased weediness potential of MON 88302 compared to conventional canola. In addition, APHIS has proposed to amend 7 CFR § 340 to include its noxious weed authority. MON 88302 would not be considered a "noxious weed" as defined by the Plant Protection Act because the data in this petition show that it has no potential to cause direct injury or damage (physical harm) to any protected interest. Therefore, Monsanto Company requests a determination from APHIS that MON 88302 and any progeny derived from crosses between MON 88302 and other commercial canola be granted non-regulated status under 7 CFR § 340.

X. ADVERSE CONSEQUENCES OF INTRODUCTION

Monsanto is not aware of any study results or observations associated with MON 88302 or the CP4 EPSPS protein indicating that there would be an adverse environmental consequence from the introduction of MON 88302. MON 88302 utilizes an improved promoter sequence to enhance CP4 EPSPS expression in male reproductive tissues (*i.e.*, pollen), compared to the promoter used to drive CP4 EPSPS production in the first-generation product, Roundup Ready canola (RT73). Enhanced CP4 EPSPS expression in the male reproductive tissues of MON 88302 allows the greater flexibility of glyphosate herbicide applications possible with MON 88302. Glyphosate is a systemic herbicide and is translocated in the plant, generally from a strong source tissue (*e.g.,* leaf) to rapidly developing, or sink tissue. Sink tissues, such as pollen, that accumulate glyphosate and lack sufficient CP4 EPSPS expression are considered to be at risk for glyphosate injury. MON 88302 plants can be sprayed with higher rates of glyphosate and at later stages of development with no detectable impact to male fertility. By virtue of enhanced CP4 EPSPS expression in male reproductive tissues, MON 88302 provides tolerance to glyphosate during the sensitive reproductive stages of growth, and enables the application of glyphosate at higher rates and at later stages of development than is possible with the current product. The CP4 EPSPS protein produced in MON 88302 is identical to the CP4 EPSPS protein present in Roundup Ready crop products that were previously granted a determination of nonregulated status by APHIS, and have been widely planted in the U.S. and globally. As demonstrated by field results and laboratory tests, the only phenotypic difference between MON 88302 and conventional canola is glyphosate tolerance.

The data and information presented in this petition demonstrate that MON 88302 is unlikely to pose an increased plant pest risk or to have an adverse environmental consequence compared to conventional canola. This conclusion is reached based on multiple lines of evidence developed from a detailed characterization of the product compared to conventional canola, followed by risk assessment on detected differences. The characterization evaluation included molecular analyses, which confirmed the insertion of a single functional copy of the CP4 EPSPS expression cassette at a single locus within the canola genome. Additionally, protein expression analyses demonstrate the CP4 EPSPS protein is expressed in vegetative and male reproductive tissues. The CP4 EPSPS protein produced by MON 88302 is similar and functionally identical to endogenous plant EPSPS enzymes, but has a much reduced affinity for glyphosate, relative to endogenous plant EPSPS. The amino acid sequence of the CP4 EPSPS protein expressed in MON 88302 is identical to the amino acid sequence of the recombinant *E. coli*-produced CP4 EPSPS protein standard previously utilized. Analyses of key nutrients, anti-nutrients and toxicants of MON 88302 seed demonstrate that MON 88302 is compositionally equivalent to conventional canola. The phenotypic evaluations of MON 88302, including an assessment of seed germination and dormancy characteristics, plant growth and development characteristics, pollen characteristics and environmental interactions also indicated MON 88302 is unchanged compared to conventional canola. There is no indication that MON 88302 would have an adverse impact on beneficial or non-target organisms, including threatened or endangered species. Therefore, based on the lack of increased pest potential or adverse environmental consequences compared to

conventional canola, the risks for humans, animals, and other NTOs from MON 88302 are negligible under the conditions of use.

The introduction of MON 88302 will not adversely impact cultivation practices or the management of weeds, diseases and insects in canola production systems. Growers familiar with the Roundup Ready canola system would continue to employ the same crop rotational practices, weed control practices and/or volunteer control measures currently in place for the first-generation Roundup Ready canola product.

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APPENDICES

Appendix A: USDA Notifications

Field trials of MON 88302 have been conducted in the U.S. since 2005. The protocols for these trials include field performance, breeding and observation, agronomics, and generation of field materials and data necessary for this petition. In addition to the MON 88302 phenotypic assessment data, observational data on pest and disease stressors were collected from these product development trials. The majority of the final reports have been submitted to the USDA. However, some final reports, mainly from the 2010 season, are still in preparation. A list of trials conducted under USDA notifications and the status of the final reports for these trials are provided in Table A-1.

Table A-1. USDA Notifications Approved for MON 88302 and Status of Trials Conducted under These Notifications

USDA Number	Effective Date	Release Site (State)	Trial Status
2005			
05-021-10n	2/28/2005	WA	Submitted to USDA
05-041-07n	4/1/2005	MN, ND (2)	Submitted to USDA
05-213-05n	8/31/2005	CA	Submitted to USDA
2006			
06-053-06n	4/7/2006	MN (3), ND (3)	Submitted to USDA
06-054-09n	4/7/2006	ND(2)	Submitted to USDA
06-055-07n	5/15/2006	MN (2), MT, ND (3)	Submitted to USDA
2008			
08-016-111n	2/15/2008	MT, ND (5)	Submitted to USDA
08-016-101n	2/20/2008	MN (5), ND (5)	Submitted to USDA
08-051-102n	3/21/2008	WA (3)	Submitted to USDA
2009			
09-034-111n	3/5/2009	ND (2)	Submitted to USDA
09-044-107n	3/15/2009	ND (2)	Submitted to USDA
09-048-105n	3/19/2009	MN (3), ND, SD (2)	Submitted to USDA
09-058-121n	3/29/2009	MN (4), ND (3)	Submitted to USDA
09-064-109n	4/4/2009	MN, ND (2)	Submitted to USDA
09-069-101n	4/9/2009	ND	Submitted to USDA
09-076-101n	4/16/2009	ID, MN (2), ND	Submitted to USDA
09-079-101n	4/19/2009	ID (3), MN, ND (3), NJ	Submitted to USDA
09-215-104n	9/2/2009	MN (2), ND	Submitted to USDA
09-257-102n	10/14/2009	CA	Submitted to USDA
2010			
10-042-102n	3/12/2010	MN	In Progress
10-050-112n	3/21/2010	MN (2), ND, SD	In Progress
10-055-104n	3/26/2010	MN, ND	In Progress
10-061-101n	3/31/2010	MN (4), ND (6)	In Progress

Appendix B: Materials, Methods, and Results for Molecular Analyses of MON 88302

B.1. Materials

The genomic DNA used in molecular analyses was isolated from leaf tissue of the R_3 generation of MON 88302 and the conventional control (Ebony). The leaf tissue was harvested from a greenhouse production in 2009. For generational stability analysis, genomic DNA was extracted from leaf tissue of the R_2, R_3, R_4, R_{5a} and R_{5b} generations of MON 88302. The leaf tissue was harvested from production plan PPN-09-523. The reference substance, PV-BNHT2672 (Figure IV-1), was used as a positive hybridization control in Southern blot analyses. Probe templates generated from PV-BNHT2672 were used as additional positive hybridization controls. As additional reference standards, the 1 Kb DNA Extension Ladder and λ DNA/*Hind* III Fragments from Invitrogen (Carlsbad, CA) were used for size estimations on agarose gels and subsequent Southern blots. The 1 Kb DNA Ladder from Invitrogen was used for size estimations on agarose gels for PCR analyses.

B.2. Characterization of the Materials

The identity of the source materials was verified by methods used in molecular characterization to confirm the presence or absence of MON 88302. The stability of the genomic DNA was confirmed by observation of interpretable signals from digested DNA samples on ethidium bromide stained agarose gels and/or specific PCR products, and the samples did not appear visibly degraded on the ethidium bromide stained gels.

B.3. DNA Isolation for Southern Blot and PCR Analyses

Genomic DNA was isolated from MON 88302 leaf tissue using a modified sarkosyl method. The leaf tissue was ground to a fine powder in liquid nitrogen using a mortar and a pestle. Approximately 4 ml of ground leaf tissue was transferred to each 50 ml conical tube. Twenty milliliter of the lysis buffer (50 mM Tris HCl, 2% w/v PVP, 20 mM EDTA, 500 mM NaCl, 1.2% w/v SDS, 0.5% w/v sarkosyl, and 0.4% w/v sodium bisulfate) and 300 µg of RNase A were added to each tube. After suspending the powder in the buffer, the samples were incubated at 60-70 °C for 60-90 min with intermittent mixing. Following the completion of the incubation, the samples were allowed to come to room temperature and 20 ml of phenol/chloroform/isoamyl alcohol (PCI) [25:24:1(v/v)] were added to each sample. The samples were then mixed by inversion with hand for 2-3 minutes followed by centrifugation at 2,000 x *g* for 20-25 min at 2-8 °C to separate the phases. The upper aqueous phase was transferred to a clean tube and the PCI extraction and centrifugation process was repeated at least once followed by a chloroform extraction. The aqueous phase was transferred to a clean tube and the amount of aqueous phase was recorded. The DNA was precipitated with an equal amount of 100% ethanol and spooled into a tube with 10-12 ml of 70% ethanol to wash the DNA. The DNA was pelleted by centrifugation at 4,000 x *g* for 5 min at 2-8 °C and the 70% ethanol was discarded. After being air-dried, the DNA pellet was suspended in

appropriate amount of TE buffer (10 mM Tris HCl, 1 mM EDTA, pH8.0) and stored in a 4 ºC refrigerator or a -20 °C freezer.

B.4. Quantification of DNA

Genomic DNA was quantified using a DyNA Quant 200 Fluorometer (Hoefer, Inc., Holliston, MA). Molecular Size Marker IX (Roche, Indianapolis, IN) was used as the calibration standard.

B.5. Restriction Enzyme Digestion of DNA

Approximately ten micrograms (µg) of genomic DNA extracted from MON 88302 and conventional control were digested with restriction enzyme *Ase* I (New England Biolabs, Inc. Ipswich, MA) and a combination of restriction enzymes *Sal* I and *Sca* I (New England Biolabs, Inc.). All digests were conducted in 1X NEBuffer 3 (New England Biolabs, Inc.) at 37°C in a total volume of ~500 microliter (µl) with ~50 units of each restriction enzyme. Digests conducted with the combination of restriction enzymes *Sal* I and *Sca* I also included 1X BSA (New England Biolabs, Inc.) in the reaction. For the purpose of running positive hybridization controls, ~10 µg of genomic DNA extracted from the conventional control was digested with the restriction enzyme *Ase* I and the appropriate positive hybridization control(s) were added to these digests prior to loading the agarose gel.

B.6. Agarose Gel Electrophoresis

Digested DNA was resolved on ~0.8% (w/v) agarose gels. For T-DNA insert/copy number and backbone analyses, individual digests containing ~10 µg each of MON 88302 and conventional control genomic DNA were loaded on the same gel in a long run/short run format. The long run allows for greater resolution of large molecular weight DNA, whereas the short run allows for retaining the small molecular weight DNA on the gel. The positive hybridization controls were only run in the short run format. For the insert stability analysis, individual digests of ~10 µg each of genomic DNA extracted from five leaf samples from four generations of MON 88302 and the conventional control along with the positive hybridization controls were loaded on the agarose gel in a single run format.

B.7. DNA Probe Preparation for Southern Blot Analyses

Probe templates were prepared by PCR amplification using the PV-BNHT2672 DNA as a template. The PCR products were separated on an agarose gel by electrophoresis and purified from the gel using QIAquick Gel Extraction Kit (Qiagen, Valencia, CA) according to manufacturer's instruction. The probe templates were designed based on the nucleotide composition (%GC) of the sequence in order to optimize the detection of DNA sequences during hybridization. When possible, probes possessing similar melting temperature (Tm) were combined in the same Southern blot hybridization. Approximately 25 ng of each probe template were radiolabeled with either [α-^{32}P] deoxycytidine triphosphate (dCTP) (6000 Ci/mmol) or [α-^{32}P] deoxyadenosine

triphosphate (dATP) (6000 Ci/mmol) using RadPrime DNA Labeling System (Invitrogen, Carlsbad, CA) according to manufacturer's instruction

B.8. Southern Blot Analyses of DNA

Genomic DNA isolated from MON 88302 and the conventional control was digested and evaluated using Southern blot analyses (Southern, 1975). The PV-BNHT2672 DNA, previously digested with the combination of restriction enzymes *Bam* HI and *Sca* I was added to conventional control genomic DNA digested with *Ase* I to serve as positive hybridization control on each Southern blot. When multiple probes were hybridized simultaneously to one Southern blot, the probe templates were spiked in the digested conventional control genomic DNA to serve as additional positive hybridization controls on the Southern blot. The DNA was then separated by agarose gel electrophoresis and transferred onto a nylon membrane. Southern blots were hybridized and washed at 55 °C, 60 °C, or 65 °C, depending on the calculated melting temperature (Tm) of the probes that were used. Table A-1 lists the radiolabeling conditions and hybridization temperatures of the probes used in this study. Multiple exposures of each blot were then generated using Kodak Biomax MS film (Eastman Kodak, Rochester, NY) in conjunction with one Kodak Biomax MS intensifying screen in a -80 °C freezer.

Table B-1. Hybridization Conditions of Utilized Probes

Probe	DNA Probe	Element Sequence Spanned by DNA Probe	Probe labeled with dNTP (^{32}P)	Hybridization/Wash Temperature (°C)
1	T-DNA Probe 1	B-Right Border, P-*FMV/Tsf1*, L-*Tsf1*, I-*Tsf1*, TS-*CTP2* (portion)	dATP	55
2	T-DNA Probe 2	TS-*CTP2* (portion), CS-*cp4 epsps* (portion)	dATP	60
3	T-DNA Probe 3	CS-*cp4 epsps* (portion), T-*E9,* B-Left Border	dATP	55
4	Backbone Probe 4	Backbone sequence	dCTP	65
5	Backbone Probe 5	Backbone sequence	dCTP	60
6	Backbone Probe 6	Backbone sequence	dCTP	60

B.9. DNA Sequence Analyses of the Insert

Overlapping PCR products, denoted as Product A and Product B, were generated that span the insert and adjacent 5' and 3' flanking genomic DNA sequences in MON 88302. These products were sequenced to determine the nucleotide sequence of the MON 88302 insert as well as the nucleotide sequence of the genomic DNA flanking the 5' and 3' ends of the insert.

The PCR analyses were performed according to SOP BR-ME-0486-01. To generate both Product A and Product B, the PCR reactions were conducted using 30 ng of genomic DNA template in a 50 µl reaction volume containing a final concentration of 1.5 mM $MgSO_4$ (Novagen, Madison, WI), 0.3 µM of each primer, 0.2 mM of each dNTP (Novagen, Madison, WI), and 2.0 units of KOD Hot Start DNA polymerase (Novagen, Madison, WI). For the generation of Product B, a final concentration of 1 M betaine (USB Corp. Cleveland, OH) was also included in the reaction. The amplification of Product A was performed under the following cycling conditions: 1 cycle at 94 ºC for 2 minutes; 35 cycles at 94 ºC for 30 seconds, 65 ºC for 15 seconds, 72 ºC for 2 minutes; 1 cycle at 72 ºC for 5 minutes. The amplification of Product B was performed under the following cycling conditions: 1 cycle at 94 ºC for 2 minutes; 35 cycles at 94 ºC for 30 seconds, 60 ºC for 15 seconds, 72 ºC for 2 minutes; 1 cycle at 72 ºC for 5 minutes.

Aliquots of each PCR product were separated on 1.0% (w/v) agarose gels and visualized by ethidium staining to verify that the products were of the expected size. Prior to sequencing, each verified PCR product was purified with the QIAquick PCR Purification Kit (Qiagen, Inc., Valencia, CA) according to manufacturer's instruction and gel quantified according to SOP BR-ME-1222-01. The purified PCR products were sequenced using multiple primers, including primers used for PCR amplification. All sequencing was performed by the Monsanto Genomics Sequencing Center using BigDye terminator chemistry (Applied Biosystems, Foster City, CA).

Numerous sequencing reactions performed on the overlapping PCR products were compiled and a consensus sequence was generated. This consensus sequence was aligned with the sequence of plasmid vector PV-BNHT2672 to determine the integrity and genomic organization of the integrated DNA and the junctions of the 5' and 3' flanks in MON 88302.

B.10. PCR and DNA Sequence Analysis to Examine the MON 88302 Insertion Site

To determine the integrity and genomic organization of the insertion site in MON 88302 and to demonstrate that the DNA sequences flanking the insert in MON 88302 are native to the canola genome, PCR analyses were performed on the genomic DNA from both MON 88302 and the conventional control Ebony. The primers used in this analysis were designed from the genomic DNA sequences flanking the insert in MON 88302. A forward primer specific to the genomic DNA sequence flanking the 5' end of the insert was paired with a reverse primer specific to the genomic DNA sequence flanking the 3' end of the insert.

The PCR analyses were performed according to SOP BR-ME-0486-01. The PCR reactions were conducted using 30 ng of genomic DNA template in a 50 µl reaction volume containing a final concentration of 2 mM $MgSO_4$, 0.4 µM of each primer, 0.2 mM of each dNTP, and 2.0 units of KOD Hot Start DNA polymerase. The amplification was performed under the following cycling conditions: 1 cycle at 94ºC for 2 minutes; 35 cycles at 94ºC for 15 seconds, 64ºC for 30 seconds, 72ºC for 2 minutes; 1 cycle at 72ºC for 5 minutes.

Aliquots of each PCR product were separated on 1.0% (w/v) agarose gels and visualized by ethidium staining to verify that a unique PCR product was produced from MON 88302 or the conventional control Ebony genomic DNA. Prior to sequencing, only the verified PCR product from the conventional control Ebony was purified with the QIAquick PCR Purification Kit according to manufacturer's instruction and gel quantified according to SOP BR-ME-1222-01. The purified PCR product was sequenced using multiple primers, including primers used for PCR amplification. All sequencing was performed by the Monsanto Genomics Sequencing Center using BigDye terminator chemistry.

Numerous sequencing reactions performed on the PCR product were compiled and a consensus sequence was generated. This consensus sequence was aligned with the 5' and 3' flanking sequences of the MON 88302 insert to determine the integrity and genomic organization of the insertion site in MON 88302.

References for Appendix B

Southern, E.M. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. Journal of Molecular Biology 98: 503-517.

Appendix C: Materials, Methods, and Results for Characterization of CP4 EPSPS Protein Produced in MON 88302

C.1. Materials

The MON 88302-produced CP4 EPSPS protein (lot 11266369) was purified from seed of MON 88302 (lot 11225246). The MON 88302-produced CP4 EPSPS protein was stored in a -80 ºC freezer in a buffer solution containing 50 mM Tris-HCl, pH 7.5, 50 mM KCl, 2 mM dithiothreitol (DTT), 1 mM benzamidine-HCl, and 25% glycerol.

The *E. coli*-produced CP4 EPSPS protein (lot 10000739) was used as the reference substance. The CP4 EPSPS protein reference substance was generated from cell paste produced by large-scale fermentation of *E. coli* containing the pMON21104 expression plasmid. The coding sequence for *cp4 epsps* contained on the expression plasmid (pMON21104) was confirmed prior to and after fermentation. The *E. coli*-produced CP4 EPSPS protein was previously characterized.

C.2. Description of Assay Controls

Protein MW standards (Precision Plus Protein Standards Dual color; Bio-Rad, Hercules, CA) were used to calibrate some SDS-PAGE gels and verify protein transfer to polyvinylidene difluoride (PVDF) and nitrocellulose membranes. Broad Range SDS-PAGE molecular weight standards (Bio-Rad, Hercules, CA) were used to generate a standard curve for the apparent MW estimation. The *E. coli*-produced CP4 EPSPS reference standard was used to construct a standard curve for the estimation of total protein concentration using a Bio-Rad protein assay. A phenylthiohydantoin (PTH) amino acid standard mixture (Applied Biosystems, Foster City, CA) was used to calibrate the instrument for each analysis. A peptide mixture (Sequazyme Peptide Mass Standards kit, Applied Biosystems, Foster City, CA) was used to calibrate the MALDI-TOF mass spectrometer for tryptic mass and a bovine serum albumin (BSA) standard (NIST, Gaithersburg, MD) was used to calibrate the MALDI-TOF mass spectrometer for intact mass analysis. Transferrin (Sigma-Aldrich, St. Louis, MO) was used as positive control for glycosylation analysis.

C.3. CP4 EPSPS Protein Purification

The plant-produced CP4 EPSPS protein was purified from seed of MON 88302. The purification procedure was not performed under a GLP plan; however, all procedures were documented on worksheets and, where applicable, SOPs were followed. The CP4 EPSPS protein was purified at ~4 °C from an extract of ground seed using a combination of ammonium sulfate fractionation, hydrophobic interaction chromatography, anion exchange chromatography, and cellulose phosphate affinity chromatography. The purification procedure is briefly described below.

Approximately 500 g of seed of MON 88302 was frozen with liquid nitrogen in a mortar and ground with a pestle. The partially crushed seed was further ground using a Magic Bullet grinder. The ground seed was then defatted by extraction with heated hexane

(~50 °C) followed by vacuum filtration. This was repeated three times at a ground seed (g) to hexane volume (ml) ratio of approximately 1:5. The defatted ground seed was allowed to dry overnight at room temperature in a fume hood. The following day the defatted ground seed was mixed with extraction buffer (100 mM Tris-HCl, pH 7.5, 2 mM EDTA, 2 mM benzamidine-HCl, 4 mM DTT, 1 mM phenylmethylsulfonyl fluoride, 1% polyvinylpolypyrrolidone, and 10% glycerol) for ~1.5 h at a sample weight (g) to buffer volume (ml) ratio of approximately 1:10. The slurry was centrifuged at 15,182 x *g* for 1 h at ~ 4 ºC. The supernatant (~3.5 liters) was collected and brought to 45% ammonium sulfate saturation by slow addition of 903 g of ammonium sulfate in a cold room (~4 ºC). The solution was stirred for ~1 h at ~4 ºC and then centrifuged at 15,182 x *g* for 1 h. The supernatant (~3.8 liters) was again collected and 592 g of ammonium sulfate was added to bring the solution to 70% ammonium sulfate saturation. The solution was stirred for ~1 h in a cold room and the pellet was collected by centrifugation at 15,182 x *g* for 1 h. The pellet was re-suspended in 1 liter of PS(A) buffer [50 mM Tris-HCl, pH 7.5, 1 mM DTT, 10% (v/v) glycerol,1.5 M ammonium sulfate]. The sample was loaded onto a 460 ml column (5 cm x 23 cm) of Phenyl Sepharose Fast Flow (GE Healthcare, Piscataway, NJ) equilibrated with PS(A) buffer. Proteins were eluted with a linear salt gradient that decreased from 1.5 M to 0 M ammonium sulfate over a volume of 2.3 liters. Fractions containing the CP4 EPSPS protein, identified based on immunoblot analysis and SDS-PAGE analysis, were pooled to a final volume of ~440 ml. The pooled sample was desalted by dialysis against 20 liters of QS(A) buffer (50 mM Tris-HCl, pH 7.5, 1 mM EDTA, 1 mM benzamidine-HCl, 4 mM DTT) at ~4 °C using a dialysis tubing [Spectrum Laboratories, Inc., Rancho Dominguez, CA; Molecular Weight Cutoff (MWCO): 3.5 kDa] for a total of 16 h.

The desalted sample (600 ml) was loaded onto a 180 ml column (5 cm x 9.2 cm) of Q Sepharose Fast Flow anion exchange resin (GE Healthcare, Piscataway, NJ) equilibrated with QS(A) buffer. The bound CP4 EPSPS was eluted with a linear salt gradient that increased from 0 M to 0.4 M KCl in QS(A) buffer over 2.1 liters. Fractions containing CP4 EPSPS, identified by immunoblot analysis, were pooled to a final volume of ~ 280 ml. The pooled sample was dialyzed against 20 liters CP(A) buffer (10 mM sodium citrate, pH 5.0, 1 mM benzamidine-HCl, 2 mM DTT) for a total of 18 h at ~4 °C using a dialysis tubing (Spectrum Laboratories, Inc. Rancho Dominguez, CA; MWCO: 3.5 kDa).

Of the 350 ml recovered after dialysis, approximately 50 ml of the dialyzed sample was loaded onto a 5 ml column (1.6 x 2.5 cm) of cellulose phosphate P11 cation exchange (Whatman, Kent, UK) pre-equilibrated with CP(A) buffer. After an initial wash with 40 ml of CP(A) buffer, the column was washed with 50 ml of CP(B) buffer [CP(A) buffer with pH adjusted to 5.2 and supplemented with 0.5 mM phosphoenolpyruvate (PEP)]. The column was further washed with CP(C) buffer [CP(A) buffer with pH adjusted to 5.4 and supplemented with 0.5 mM PEP]. The bound CP4 EPSPS protein was eluted over 90 ml of CP(D) buffer [CP(A) buffer with pH adjusted to 5.7 and supplemented with 0.5 mM PEP and 0.5 mM shikimate-3-phosphate (S3P)]. Fractions containing CP4 EPSPS protein, based on SDS-PAGE analysis and confirmed by immunoblot analysis, were pooled (~22 ml), supplemented with 10% glycerol, labeled Pool 1, and stored at -20 °C. Approximately 200 ml of the remaining dialyzed sample

was then loaded onto a freshly prepared 20 ml column (2.6 x 3.7 cm) of cellulose phosphate P11 cation exchange (Whatman, Kent, UK) pre-equilibrated with freshly prepared CP(A) buffer. After an initial wash with 200 ml of CP(A) buffer, the column was washed with 160 ml of freshly prepared CP(B) buffer. The column was further washed with freshly prepared CP(C) buffer. The bound CP4 EPSPS protein was eluted with freshly prepared CP(D) buffer. Fractions containing CP4 EPSPS protein, based on SDS-PAGE analysis and confirmed by immunoblot, were pooled (Pool 2). Pool 1 and Pool 2 were combined (~82 ml) and divided between four iCon™ concentrators (MWCO: 20 kDa; size: 20 ml; Pierce, Rockford, IL) and concentrated by centrifugation at 4,000 x *g* for 30 min at ~4 °C. Buffer exchange was carried out in the same concentrators by the addition of ~19 ml an initial buffer containing 50 mM Tris-HCl, pH 7.5, 50 mM KCl, 2 mM DTT, 1 mM benzamidine-HCl followed by centrifugation at 4,000 x *g* for 30 min at ~4 °C repeated four times. After the fourth buffer exchange the remaining sample (~10 ml) was transferred to a new iCon concentrator (MWCO: 20 kDa; size: 20 ml; Pierce, Rockford, IL), supplemented with equal volume of the buffer containing 50 mM Tris-HCl pH 7.5, 50 mm KCl, 2 mm DTT, 50% glycerol and 1 mm benzamidine-HCl, and the sample was concentrated to ~2.4 ml. The final buffer composition of the sample was: 50 mM Tris-HCl, pH 7.5, 50 mM KCl, 2 mM DTT, 1 mM benzamidine-HCl and 25% glycerol. This CP4 EPSPS protein purified from the seed of MON 88302 was aliquoted and stored in a -80 °C freezer.

C.4. N-Terminal Sequencing

C.4.1. Methods

N-terminal sequencing, carried out by automated Edman degradation chemistry, was used to confirm the identity of the MON 88302-produced CP4 EPSPS.

MON 88302-produced CP4 EPSPS was separated by SDS-PAGE and transferred to a PVDF membrane. The blot was stained using Coomassie Blue R-250. The major band at ~44 kDa containing the test protein was excised from the blot and was used for N-terminal sequence analysis. The analysis was performed for 15 cycles using automated Edman degradation chemistry (Hunkapiller et al., 1983). An Applied Biosystems 494 Procise Sequencing System with a 140C Microgradient pump and a 785 Programmable Absorbance Detector was controlled with Procise® Control (version 1.1a) software. Chromatographic data were collected using Atlas 2003 software (version 3.59a, LabSystems, Altrincham, Cheshire, England). A control protein, β-lactoglobulin, (Applied Biosystems, Foster City, CA) was analyzed before and after the sequence analysis of the CP4 EPSPS protein to verify that the sequencer met performance criteria for repetitive yield and sequence identity. Identity was established if ≥ 8 amino acids, consistent with the predicted sequence of the N-terminus of the MON 88302-produced CP4 EPSPS, were observed during analysis.

C.4.2. Results of the N-terminal Sequence Analysis

N-terminal sequencing of the first 15 amino acids was performed on MON 88302-produced CP4 EPSPS protein. The expected sequence for the CP4 EPSPS protein deduced from the *cp4 epsps* gene present in MON 88302 was observed. The data obtained correspond to the deduced CP4 EPSPS protein beginning at amino acid positions 2 and 4 (Table C-1, Experimental Sequence 1 and 2, respectively). Hence, the sequence information confirms the identity of the CP4 EPSPS protein isolated from the seed of MON 88302.

Amino acid residue # from the N-terminus	→	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Expected Sequence	→	M	L	H	G	A	S	S	R	P	A	T	A	R	K	S	S	G	L
Experimental Sequence 1	→	-	L	H	G	A	X	X	X	P	A	T	X	X	X	X	X	^	^
Experimental Sequence 2	→	-	-	-	G	A	S	X	R	P	A	T	A	X	K	S	X	G	X

Table C-1. N-Terminal Sequence of the MON 88302-produced CP4 EPSPS
The expected amino acid sequence of the N-terminus of CP4 EPSPS protein was deduced from the *cp4 epsps* coding region present in MON 88302. The experimental sequences obtained from the MON 88302-produced CP4 EPSPS protein were compared to the expected sequence. The single letter IUPAC-IUB amino acid code is M, methionine; L, leucine; H, histidine; G, glycine; A, alanine, S, serine; R, arginine; P, proline; T, threonine; K. lysine; (X) indicates that the residue was not identifiable; (-) indicates the residue was not observed; (^) indicates not done, *i.e.*, sequencing cycle was not conducted.

C.5. MALDI-TOF Tryptic Mass Map Analysis

C.5.1. Methods

MALDI-TOF tryptic mass fingerprint analysis was used to confirm the identity of the MON 88302-produced CP4 EPSPS protein. MON 88302-produced CP4 EPSPS protein was subjected to SDS-PAGE and the gel was stained using Brilliant Blue G Colloidal stain. Each ~44 kDa band was excised and transferred to a microcentrifuge tube. The gel bands were washed in 100 mM ammonium bicarbonate and then, to reduce the protein in each, gel bands were incubated in 100 µl of 10 mM DTT at ~37°C for 2 h. The protein was then alkylated in the dark for 25 min with 100 µl of 20 mM iodoacetic acid and washed with 200 µl of 25 Mm ammonium bicarbonate for 3 x 20 min washes. Gel bands were dried with a Speed-Vac® concentrator (Thermo Fisher Scientific, Waltham, MA) and then rehydrated with 20 µl of trypsin solution (20 µg/ml). After 1 h, excess liquid was removed and the gel was incubated at ~37 °C for 16 h in 40 µl of 10% acetonitrile in

25 mM ammonium bicarbonate. Gel bands were sonicated for 5 min to further elute proteolytic fragments. The resulting extracts were transferred to new microcentrifuge tubes labeled Extract 1 and dried using Speed-Vac concentrator. The gel bands were re-extracted twice with 30 µl of a 60% acetonitrile, 0.1% trifluoroacetic acid, 0.1% β-octyl-glucopyranoside solution and sonicated for 5 min. Both 60% acetonitrile, 0.1% trifluoroacetic acid, 0.1% β-octyl-glucopyranoside extracts were pooled into a new tube labeled Extract 2 and dried with a Speed-Vac concentrator. A solution of 0.1% trifluoroacetic acid (TFA) was added to all Extract 1 and 2 tubes and they were dried as before. To acidify the extracts, a solution of 50% acetonitrile, 0.1% TFA was added to each tube and all were sonicated for 5 min. Each extract (0.3 µl) was spotted to three wells on an analysis plate. For each extract 0.75 µl of 2, 5-dihydroxybenzoic acid (DHB), α-cyano-4-hydroxycinnamic acid (α Cyano), or 3,5-dimethoxy-4-hydroxycinnamic acid (sinapinic acid) (Waters Corp., Milford, MA) was added to one of the spots. The samples in DHB matrix were analyzed in the 300 to 7000 Da range. Samples in α-Cyano and Sinapinic acid were analyzed in the 500 to 5000 and 500 to 7000 Da range, respectively. Protonated peptide masses were monoisotopically resolved in reflector mode (Aebersold, 1993; Billeci and Stults, 1993). CalMix 2 was used as the external calibrant (Sequazyme Peptide Mass Standards kit, Applied Biosystems, Foster City, CA) for the analysis. GPMAW32 software (Lighthouse Data, Odense M, Denmark) was used to generate a theoretical trypsin digest of the CP4 EPSPS protein sequence. Those experimental masses within 1 Da of a theoretical mass were matched. All matching masses were tallied and a coverage map was generated for the mass fingerprint. The tryptic mass fingerprint coverage was considered acceptable if ≥ 40% of the protein sequence was identified by matching experimental masses observed for the tryptic peptide fragments to the expected masses for the fragments.

C.5.2. Results of MALDI-TOF Tryptic Mass Map Analysis

The identity of the MON 88302-produced CP4 EPSPS protein was also confirmed by MALDI-TOF MS analysis of peptide fragments produced from tryptic digestion of the MON 88302-produced CP4 EPSPS protein. The ability to identify a protein using this method is dependent upon matching a sufficient number of observed tryptic peptide fragment masses with predicted tryptic peptide fragment masses. In general, protein identification made by peptide mapping is considered to be reliable if the measured coverage of the sequence is 15% or higher with a minimum of five matched peptides (Jensen et al., 1997).

There were 34 unique peptides identified that corresponded to the masses (Table C-2) expected to be produced by tryptic digestion of the CP4 EPSPS protein. The identified masses were used to assemble a coverage map of the entire CP4 EPSPS protein (Figure C-1). The experimentally determined mass coverage of the CP4 EPSPS protein was 85.5% (389 out of 455 amino acids). This analysis serves as additional identity confirmation for the MON 88302-produced CP4 EPSPS protein.

Table C-2. Summary of the Tryptic Masses Identified for the MON 88302-produced CP4 EPSPS Using MALDI-TOF Mass Spectrometry

α-Cyano	α-Cyano	DHB	DHB	Sinapinic acid	Sinapinic acid	Expected Mass[1]	Diff[2]	Position[3]	Sequence
Extract 1	Extract 2	Extract 1	Extract 2	Extract 1	Extract 2				
		389.28				389.25	0.03	225-227	TIR
		474.32				474.27	0.05	228-231	LEGR
506.29						506.22	0.07	354-357	ESDR
		529.36				529.30	0.06	24-28	IPGDK
599.43	599.51	599.41				599.33	0.10	29-33	SISHR
616.44	616.48	616.41		616.24		616.34	0.10	128-132	RPMGR
629.44		629.45				629.29	0.15	201-205	DHTEK
629.44		629.45				629.34	0.10	383-388	GRPDGK
711.57	711.62	711.56	711.61			711.45	0.12	133-138	VLNPLR
		790.55				790.48	0.07	306-312	VRSSTLK
		790.55				790.41	0.14	139-145	EMGVQVK
		805.54				805.43	0.11	447-453	IELSDTK
835.54	835.58	835.53				835.39	0.15	62-69	AMQAMGAR
863.61	863.68	863.60				863.46	0.15	15-23	SSGLSGTVR
872.61	872.66	872.61	872.67	872.53		872.45	0.16	313-320	GVTVPEDR
872.61	872.66	872.61	872.67	872.53		872.52	0.09	358-366	LSAVANGLK
		930.66				930.51	0.15	169-177	VPMASAQVK
948.68	948.74	948.68	948.75			948.52	0.16	161-168	TPTPITYR
991.72		991.71				991.55	0.17	14-23	KSSGLSGTVR
1115.75	1115.83	1115.77	1115.86	1115.69		1115.57	0.18	295-305	LAGGEDVADLR
1357.94	1358.01	1357.97	1358.05	1357.89		1357.71	0.23	146-157	SEDGDRLPVTLR
1359.88	1359.96	1359.91	1360.00	1359.81	1359.87	1359.72	0.16	354-366	ESDRLSAVANGLK
1359.88	1359.96	1359.91	1360.00	1359.81	1359.87	1359.64	0.24	34-46	SFMFGGLASGETR
1559.11	1559.18	1559.13	1559.01			1558.83	0.28	47-61	ITGLLEGEDVINTGK
1647.10	1647.24	1647.16	1647.24			1646.84	0.26	389-405	GLGNASGAAVATHLDHR
1764.10	1764.26	1764.16		1764.06		1763.81	0.29	367-382	LNGVDCDEGETSLVVR
1994.31	1994.43	1994.35	1994.55	1994.21	1994.35	1993.97	0.34	206-224	MLQGFGANLTVETDADGVR
2183.54	2183.67	2183.57	2183.80	2183.45	2183.53	2183.17	0.37	275-294	TGLILTLQEMGADIEVINPR
2367.73	2367.87	2367.77	2367.85	2367.65	2367.8	2367.33	0.40	178-200	SAVLLAGLNTPGITTVIEPIMTR
2450.65	2450.83	2450.80		2450.51	2450.6	2450.23	0.42	24-46	IPGDKSISHRSFMFGGLASGETR
2450.65	2450.83	2450.80		2450.51	2450.6	2450.22	0.43	105-127	LTMGLVGVYDFDSTFIGDASLTK
3247.10 (Ave)	3247.05 (Ave)			3246.89 (Ave)	3246.97 (Ave)	3246.54 (Ave)	0.56	73-104	EGDTWIIDGVGNGGLLAPEAPLDFGNAATGCR
3251.94 (Ave)	3252.18 (Ave)	3252.06 (Ave)	3253.42 (Ave)	3252.58 (Ave)	3252.04 (Ave)	3251.75 (Ave)	0.19	321-351	APSMIDEYPILAVAAAFAEGATVMNGLEELR
		4191.34 (Ave)	4191.48 (Ave)	4191.89 (Ave)	4191.63 (Ave)	4190.89 (Ave)	0.37	234-274	LTGQVIDVPGDPSSTAFPLVAALLVPGSDVTILNVLMNPTR

[1]Only experimental masses that matched expected masses are listed in the table.
[2]The difference between the expected mass and the first column mass. Other masses shown within a row are also within 1 Da of the expected mass.
[3]Position refers to amino acid residues within the predicted CP4 EPSPS sequence as depicted in Figure C-1.
DHB = 5-dihydroxybenzoic acid matrix, α-cyano = α-cyano-4-hydroxycinnamic acid matrix; Sinapinic acid = 3, 5-dimethoxy-4-hydroxycinnamic acid matrix; Ave = experimental mass average (for large peptides the monoisotopic mass is poorly resolved, therefore the mass average value is used for comparison).

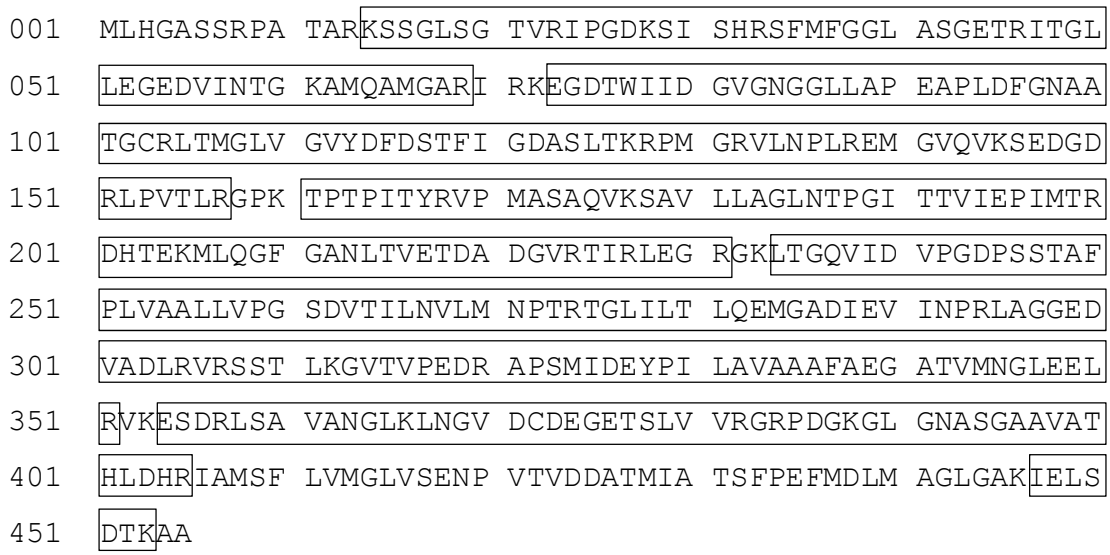

Figure C-1. MALDI-TOF MS Coverage Map of the MON 88302-produced CP4 EPSPS Protein
The amino acid sequence of the mature CP4 EPSPS protein was deduced from the *cp4 epsps* gene present in MON 88302. Boxed regions correspond to regions covered by tryptic peptides that were identified from the MON 88302-produced CP4 EPSPS protein sample using MALDI-TOF MS. In total, 85.5% (389 of 455 total amino acids) of the expected protein sequence was covered by the identified peptides.

C.6. Western Blot Analysis-Immunoreactivity

C.6.1. Methods

Western blot analysis was performed to confirm the identity of the CP4 EPSPS protein purified from seed of MON 88302 and to compare the immunoreactivity of the MON 88302- and *E. coli*-produced proteins.

The MON 88302- and *E. coli*-produced CP4 EPSPS proteins were analyzed concurrently on the same gel using three loadings of 1, 2 and 3 ng. Loadings of the three concentrations were made in duplicate on the gel. Aliquots of each protein were diluted in water and 5X Laemmli buffer (LB) containing 312 mM Tris-HCl, 20% (v/v) 2-mercaptoethanol, 10% (w/v) SDS, 0.025% (w/v) bromophenol blue, 50% (v/v) glycerol, pH 6.8), heated at ~99 °C for 3 min, and applied to a 15-well pre-cast Tris-glycine 4-20% polyacrylamide gradient gel (Invitrogen, Carlsbad, CA). Pre-stained molecular weight markers (Precision Plus Protein Standards Dual color; Bio-Rad, Hercules, CA) were loaded in parallel to verify electrotransfer of the proteins to the membrane and to estimate the size of the immunoreactive bands observed. Electrophoresis was performed at a constant voltage of 130 V for 90 min. Electrotransfer to a 0.45 µm nitrocellulose membrane (Invitrogen, Carlsbad, CA) was performed for 90 min at a constant voltage of 30 V. After electrotransfer, the membrane was blocked

for 1 h with 5% (w/v) non-fat dried milk (NFDM) in 1X phosphate buffered saline containing 0.05% (v/v) Tween-20 (PBST). The membrane was then probed with a 1:1000 dilution of goat anti-CP4 EPSPS antibody (lot 10000787) in 5% NFDM in PBST overnight at 4 °C. Excess antibody was removed using three 10 min washes with PBST. Finally, the membrane was probed with horseradish peroxidase (HRP)-conjugated rabbit anti-goat IgG (Thermo, Rockford, IL) at a dilution of 1:10,000 in 5% NFDM in PBST for 1 h at room temperature. Excess HRP-conjugate was removed using three 10 min washes with PBST. All washes were performed at room temperature. Immunoreactive bands were visualized using the ECL detection system (GE, Healthcare, Piscataway, NJ) with exposure (1 and 3 min) to Amersham Hyperfilm ECL (GE, Healthcare, Piscataway, NJ). The film was developed using a Konica SRX-101A automated film processor (Tokyo, Japan).

Quantification of the bands on the blot was performed using a Bio-Rad GS-800 densitometer with the supplied Quantity One software (version 4.4.0, Hercules, CA) using the lane finding and contour tools. The signal intensities of the immunoreactive bands observed for the MON 88302- and *E. coli*-produced proteins migrating at the expected position on the blot film were quantified as "contour quantity" values. The raw data was exported to a Microsoft Excel (2007) file for the pair wise comparison of the average contour quality of the load replicates. An average difference was calculated for each comparison to assess the immunoreactivity equivalence. The immunoreactivity of the MON 88302- and *E. coli*-produced CP4 EPSPS proteins were considered equivalent if the signal intensity of the CP4 EPSPS bands were within 35% of one another.

C.6.2. Results of CP4 EPSPS Protein Immunoreactivity Equivalence

Western blot analysis was conducted using goat anti-CP4 EPSPS polyclonal antibody to 1) confirm the identity of the CP4 EPSPS protein isolated from the seed of MON 88302 and 2) to determine the relative immunoreactivity of the MON 88302- and the *E. coli*-produced CP4 EPSPS proteins. The results demonstrated that the anti-CP4 EPSPS antibody recognized the MON 88302-produced CP4 EPSPS protein that migrated to an identical position as the *E. coli*-produced CP4 EPSPS protein (Figure C-2). Furthermore, the immunoreactive signal increased with increasing amounts of CP4 EPSPS protein loaded.

Densitometric analysis was conducted to compare the immunoreactivity of MON 88302- and *E. coli*-produced CP4 EPSPS proteins**.** The average signal intensity (OD x mm2) from the MON 88302-produced CP4 EPSPS bands and the *E. coli*-produced CP4 EPSPS bands at each amount of protein loaded are shown in Table C-3. The percent differences in the average signal intensity from the MON 88302-produced CP4 EPSPS bands and from the *E. coli*-produced CP4 EPSPS bands for each amount analyzed was calculated. These values as well as the overall average percent difference (24.1%) are also shown in Table C-3. The acceptance criterion for equivalence of immunoreactivity (±35%) of the MON 88302-produced CP4 EPSPS bands and *E. coli*-produced CP4 EPSPS bands was met. Thus, the western blot analysis established identity of the MON 88302-produced CP4 EPSPS and demonstrated that the MON 88302- and *E. coli*-produced CP4 EPSPS proteins have equivalent immunoreactivity with a CP4 EPSPS-specific antibody.

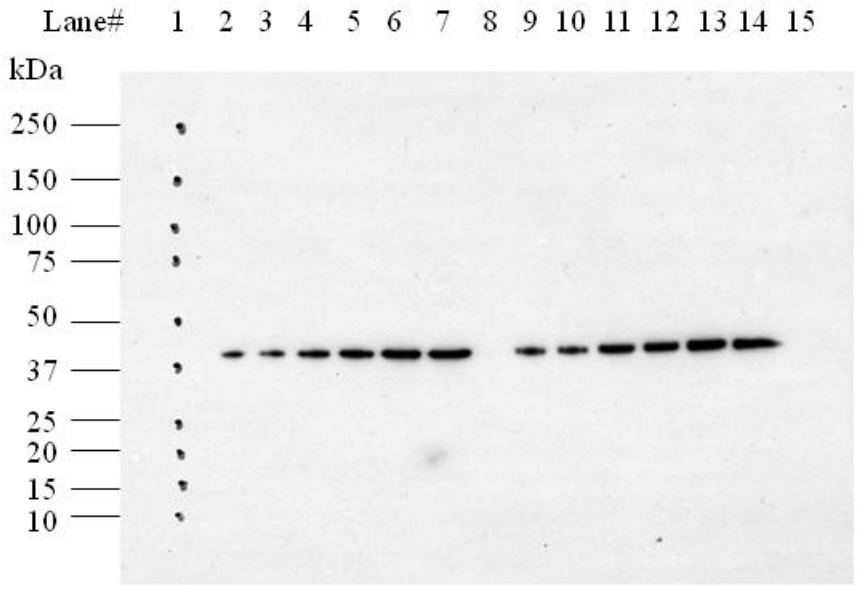

Figure C-2. Western Blot Analysis of MON 88302- and *E. coli* -produced CP4 EPSPS Proteins

Aliquots of the MON 88302-produced CP4 EPSPS protein and the *E. coli*-produced CP4 EPSPS protein were separated by SDS-PAGE and electrotransferred to a PVDF membrane. The membrane was incubated with anti-CP4 EPSPS antibodies and immunoreactive bands were visualized using an ECL system (GE Healthcare, Piscataway, NJ). Approximate molecular weights (kDa) are shown on the left and correspond to the markers loaded in lane 1. The 1 min exposure is shown.

Lane	Sample	Amount (ng)
1	Precision Plus Protein Standards Dual color	-
2	*E. coli*-produced CP4 EPSPS protein	1
3	*E. coli*-produced CP4 EPSPS protein	1
4	*E. coli*-produced CP4 EPSPS protein	2
5	*E. coli*-produced CP4 EPSPS protein	2
6	*E. coli*-produced CP4 EPSPS protein	3
7	*E. coli*-produced CP4 EPSPS protein	3
8	Empty	-
9	MON 88302-produced CP4 EPSPS protein	1
10	MON 88302-produced CP4 EPSPS protein	1
11	MON 88302-produced CP4 EPSPS protein	2
12	MON 88302-produced CP4 EPSPS protein	2
13	MON 88302-produced CP4 EPSPS protein	3
14	MON 88302-produced CP4 EPSPS protein	3
15	Empty	-

Table C-3. Comparison of Immunoreactive Signal Between MON 88302- and *E. coli*-produced EPSPS Proteins

Sample	Gel lane	Amount (ng)	Contour Qty (OD × mm^2)	Average Contour Qty[1]	Percent Difference[2] (%)	Average Difference[3] (%)
E. coli CP4 EPSPS	2	1	1.257	1.408	30.8	24.1
E. coli CP4 EPSPS	3	1	1.558			
MON 88302 CP4 EPSPS	9	1	2.064	2.033		
MON 88302 CP4 EPSPS	10	1	2.002			
E. coli CP4 EPSPS	4	2	3.296	3.748	26.5	
E. coli CP4 EPSPS	5	2	4.199			
MON 88302 CP4 EPSPS	11	2	4.979	5.101		
MON 88302 CP4 EPSPS	12	2	5.222			
E. coli CP4 EPSPS	6	3	6.264	6.407	14.9	
E. coli CP4 EPSPS	7	3	6.549			
MON 88302 CP4 EPSPS	13	3	7.737	7.527		
MON 88302 CP4 EPSPS	14	3	7.317			

[1]Average Contour Quantity = ∑(Contour Quantity)/2; contour quantity is average pixel density × band area.
[2]Percent Difference (%) = ((|Average Contour Quantity MON 88302–Average Contour Quantity *E. coli*|)/(Average Contour Quantity MON 88302)) × 100% .
[3]Average difference (%) = ∑ 〚% difference〛 /3.

C.7. Molecular Weight and Purity Estimation using SDS-PAGE

C.7.1. Methods

An aliquot of the MON 88302-produced CP4 EPSPS protein and an *E. coli*-produced CP4 EPSPS protein was mixed with 5X LB and diluted with H_2O to a final total protein concentration of 0.2 µg/µl. Bio-Rad broad range Molecular Weight Standards (Hercules, CA) were diluted to a final total protein concentration of 0.9 µg/µl. The MON 88302-produced CP4 EPSPS protein was analyzed in duplicate at 1, 2, and 3 µg protein per lane. The *E. coli*-produced CP4 EPSPS reference standard was analyzed at 1 µg total protein in a single lane. The samples were loaded onto a 10-well pre-cast Tris glycine 4-20% polyacrylamide gradient mini-gel (Invitrogen, Carlsbad, CA) and electrophoresis was performed at a constant voltage of 130 V for 95 min. Proteins were fixed by placing the gel in a solution of 40% (v/v) methanol and 7% (v/v) acetic acid for ~30 min, stained for ~16 h with Brilliant Blue G-Colloidal stain (Sigma-Aldrich, St. Louis, MO). Gels were destained for 30 to 45 sec with a solution containing 10% (v/v) acetic acid and 25% (v/v) methanol, and for ~7 h with 25% (v/v) methanol. Analysis of the gel was performed using a Bio-Rad GS-800 densitometer with the supplied Quantity One software (version 4.4.0, Hercules, CA). The apparent MW of each observed band was estimated from a standard curve generated by the Quantity One software which was based on the MWs of the markers and their migration distance on the gel. To determine purity, all visible bands within each lane were quantified using Quantity One software. The purity of the MON 88302-produced CP4 EPSPS protein was reported as the percent

of the total of all quantified bands in a lane. Apparent MW and purity were reported as an average of all six lanes containing the MON 88302-produced CP4 EPSPS

C.7.2. Results of CP4 EPSPS Protein Molecular Weight Equivalence

For molecular weight and purity analysis, the MON 88302-produced CP4 EPSPS protein was separated using SDS-PAGE. The gel was stained with Brilliant Blue G Colloidal stain and analyzed by densitometry (Figure C-3). The MON 88302-produced CP4 EPSPS protein (Figure C-3, lanes 3-8) migrated to the same position on the gel as the *E. coli*-produced CP4 EPSPS protein (Figure C-3, lane 2) and had an apparent molecular weight of 43.1 kDa (Table C-4). The apparent molecular weight of the *E. coli*-produced CP4 EPSPS protein as reported on its Certificate of Analysis was 43.8 kDa (Table C-4). The apparent molecular weights of the MON 88302- and *E. coli*-produced CP4 EPSPS proteins were considered equivalent if they were within 10% of one another. Because the experimentally determined apparent molecular weight of the MON 88302-produced CP4 EPSPS protein was within 10% of the *E. coli*-produced CP4 EPSPS protein (Table C-4), the MON 88302- and *E. coli*-produced CP4 EPSPS proteins were determined to have equivalent apparent molecular weights.

The purity of the MON 88302-produced CP4 EPSPS protein was calculated based on the six loads on the gel (Figure C-3, lanes 3 to 8). The average purity was determined to be 99%.

Table C-4. Molecular Weight Comparison Between the MON 88302- and *E. coli*-produced CP4 EPSPS Proteins

Molecular Weight of MON 88302-Produced CP4 EPSPS Protein	Molecular Weight of *E. coli*-Produced CP4 EPSPS Protein[1]	% Difference from *E. coli*-Produced CP4 EPSPS Protein
43.1 kDa	43.8 kDa	1.6%

[1]The molecular weight of the *E. coli*-produced CP4 EPSPS protein as reported on its Certificate of Analysis.

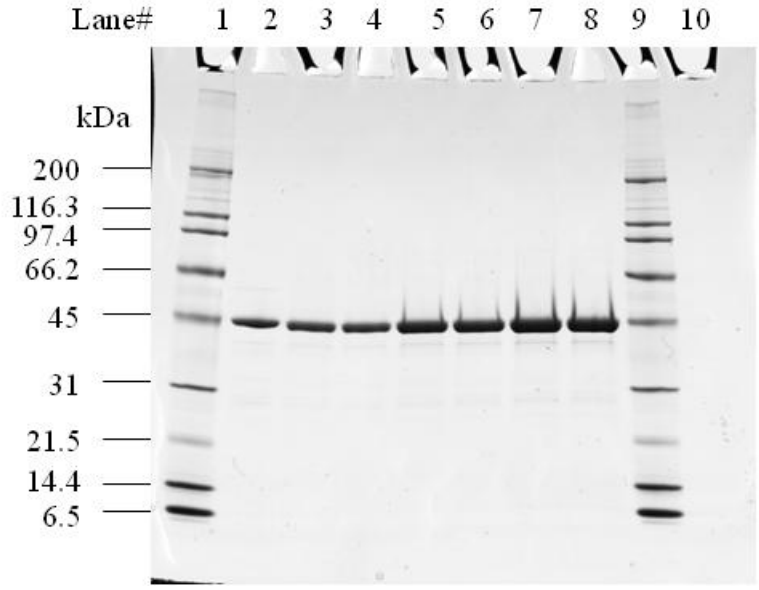

Figure C-3. Molecular Weight and Purity Analysis of the MON 88302-produced EPSPS Protein

Aliquots of the MON 88302- and the *E. coli*-produced CP4 EPSPS proteins were separated on a 4-20% Tris glycine polyacrylamide gradient gel and then stained with Brilliant Blue G-Colloidal stain. Approximate molecular weights are shown on the left and correspond to the markers loaded in Lanes 1 and 9.

Lane	Sample	Amount (µg)
1	Broad Range Molecular Weight Markers	4.5
2	*E. coli*-produced CP4 EPSPS protein	1
3	MON 88302-produced CP4 EPSPS protein	1
4	MON 88302-produced CP4 EPSPS protein	1
5	MON 88302-produced CP4 EPSPS protein	2
6	MON 88302-produced CP4 EPSPS protein	2
7	MON 88302-produced CP4 EPSPS protein	3
8	MON 88302-produced CP4 EPSPS protein	3
9	Broad Range Molecular Weight markers	4.5
10	Empty	-

C.8. Glycosylation Analysis

C.8.1. Methods

Glycosylation analysis was used to determine whether the MON 88302-produced CP4 EPSPS was post-translationally modified with covalently bound carbohydrate moieties. Aliquots of the MON 88302-produced CP4 EPSPS protein, the *E. coli*-produced CP4 EPSPS and the positive control, transferrin (Sigma-Aldrich, St Louis, MO), were each diluted with water and mixed with 1X LB. These samples were heated at ~95 °C for 3 min. The MON 88302- and the *E. coli*- produced CP4 EPSPS proteins were loaded at approximately 100 and 200 ng per lane and transferrin was loaded at approximately 50, 100, 150 and 200 ng on a Tris-glycine 10-well 4- 20% polyacrylamide gradient mini-gel (Invitrogen, Carlsbad, CA). Precision Plus Protein Dual color Standards (Bio-Rad, Hercules, CA) were also loaded to verify electrotransfer of the proteins to the membrane and as markers for molecular weight. Electrophoresis was performed at a constant voltage of 155 V for 75 min. Electrotransfer to a 0.45 µm PVDF membrane (Invitrogen, Carlsbad, CA) was performed for 35 min at a constant voltage of 100 V.

Carbohydrate detection was performed directly on the PVDF membrane at room temperature using the Amersham ECL glycoprotein Detection Module (GE, Healthcare, Piscataway, NJ). With this module, carbohydrate moieties of proteins are oxidized with sodium metaperiodate and are then biotinylated with biotin-X-hydrazide. The biotinylated proteins can be detected on the blot by addition of streptavidin conjugated to HRP for luminol-based detection using ECL reagents (GE, Healthcare, Piscataway, NJ) and with subsequent exposure (1, 2 and 3 min) to Amersham Hyperfilm (GE, Healthcare). The film was developed using a Konica SRX-101A automated film processor (Tokyo, Japan).

A second identical blot run in parallel to that used for the glycosylation analysis was stained to visualize the proteins present on the membrane. Proteins were stained for 30 sec to 2 min using Coomassie Brilliant Blue R-250 staining solution (Bio-Rad, Hercules, CA) and then destained with 1X Coomassie Brilliant Blue R-250 Destaining Solution (Bio-Rad) for 5 min. After washing with water, the blot was dried and scanned using Bio-Rad GS-800 densitometer with the supplied Quantity One software (version 4.4.0).

C.8.2. Results of Glycosylation Analysis

Some eukaryotic proteins are post-translationally modified by the addition of carbohydrate moieties (Rademacher et al., 1988). To test whether the CP4 EPSPS protein was glycosylated when expressed in the seed of MON 88302, the MON 88302-produced CP4 EPSPS protein was analyzed using an ECL Glycoprotein Detection Module (GE, Healthcare, Piscataway, NJ). Transferrin, a glycosylated protein, was used as a positive control in the assay. To assess equivalence of the MON 88302- and *E. coli*-produced CP4 EPSPS proteins, the *E. coli*-produced CP4 EPSPS protein, previously been shown to be free of glycosylation (Harrison et al., 1996), was also analyzed. The positive

control was clearly detected at expected molecular weight (~76 kDa) and the band intensity increased with increasing concentration (Figure C-4, Panel A, lanes 2-5). In contrast, signals were not observed in the lanes containing the MON 88302- or *E. coli*-produced protein at the expected molecular weight for the CP4 EPSPS protein (Figure C-4 panel A, lanes 6-9). To confirm that sufficient MON 88302- and *E. coli*-produced CP4 EPSPS proteins were present for glycosylation analysis, a second membrane (with identical loadings and transfer times) was stained with Coomassie Blue R250 for protein detection (Figure C-4 Panel B). Both the MON 88302- and *E. coli*-produced CP4 EPSPS proteins were clearly detected (Figure C-4, Panel B, Lanes 6-9). These data indicate that the glycosylation status of MON 88302-produced CP4 EPSPS protein is equivalent to that of the *E. coli*-produced CP4 EPSPS protein and that neither is glycosylated

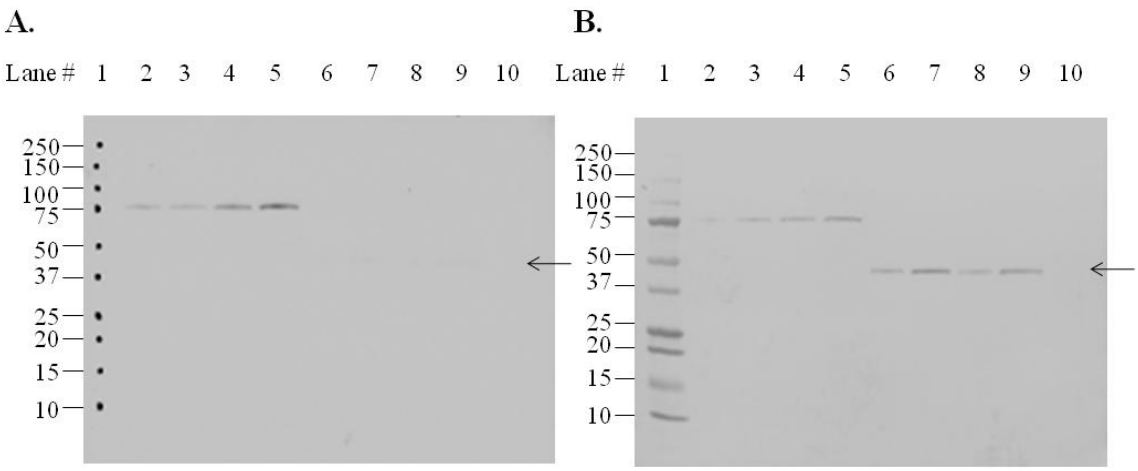

Figure C-4. Glycosylation Analysis of the MON 88302-produced CP4 EPSPS Protein

Aliquots of the transferrin (positive control), *E. coli*-produced CP4 EPSPS protein and MON 88302-produced CP4 EPSPS protein were separated by SDS-PAGE (4-20%) and electrotransferred to PVDF membranes. (A) Where present, the labeled carbohydrate moieties were detected using the ECL-based system with exposure to Hyperfilm. A 2 min exposure is shown. (B) An equivalent blot was stained with Coomassie Blue R250 to confirm the presence of proteins. The signal was captured using a Bio-Rad GS-800 with Quantity One software (version 4.4.0). Approximate molecular weights (kDa) correspond to the Precision Plus, dual color markers (used to verify transfer and MW) in Lane 1. Arrows indicate the band corresponding to CP4 EPSPS protein.

Lane	Sample	Amount (ng)
1	Precision Plus, dual color MW markers	-
2	Transferrin (positive control)	50
3	Transferrin (positive control)	100
4	Transferrin (positive control)	150
5	Transferrin (positive control)	200
6	*E. coli*-produced CP4 EPSPS (negative control)	100
7	*E. coli*-produced CP4 EPSPS (negative control)	200
8	MON 88302-produced CP4 EPSPS	100
9	MON 88302-produced CP4 EPSPS	200
10	Empty	-

C.9. Functional Activity Analysis

C.9.1. Methods

Prior to functional activity analysis, both MON 88302- and *E. coli*-produced proteins were diluted to a purity corrected concentration of ~50 µg/ml with 50 mM HEPES, pH 7.0 buffer. Assays for both proteins were conducted in triplicate. The reactions were performed in 50 mM HEPES, pH 7.0, 0.1 mM ammonium molybdate, 1 mM PEP and 5 mM potassium fluoride with or without 2 mM S3P for 2 min at ~25 °C. The reactions were initiated by the addition of PEP. After 2 min, the reactions were quenched with phosphate assay reagent (0.033% malachite green, 1.1% ammonium molybdate) and then fixed with 33% (w/v) sodium citrate. A standard curve was prepared using 0 to 10 nmoles of inorganic phosphate in water treated with the phosphate assay reagent and 33% (w/v) sodium citrate. The absorbance of each reaction and each standard was measured in duplicate at 660 nm using a PowerWave Xi™ (Bio-Tek, Richmond, VA) microplate reader. The amount of inorganic phosphate released from PEP in each reaction was determined using the standard curve. For CP4 EPSPS, the specific activity was defined in unit per mg of protein (U/mg), where a unit (U) is defined as 1 µmole of inorganic phosphate released from PEP per min at 25 °C. Calculations of the specific activities were performed using Microsoft Excel (2007).

C.9.2. Results of Functional Activity

The functional activities of the MON 88302- and *E. coli*-produced CP4 EPSPS proteins were determined using a colorimetric assay that measures formation of inorganic phosphate (Pi) from the EPSPS-catalyzed reaction between shikimate-3-phosphate (S3P) and phosphoenolpyruvate (PEP). In this assay, protein-specific activity is expressed as units per milligram of protein (U/mg), where a unit is defined as one µmole of inorganic phosphate released from PEP per minute at 25 °C. The MON 88302- and *E. coli*-produced CP4 EPSPS proteins were considered to have equivalent functional activity if the specific activities were within 2-fold of one another.

The experimentally determined specific activities for the MON 88302- and *E. coli*-produced CP4 EPSPS proteins are presented in Table C-5. The specific activities of MON 88302- and *E. coli*-produced CP4 EPSPS proteins were 4.93 U/mg and 2.79 U/mg of CP4 EPSPS protein, respectively. Because the specific activity of the MON 88302-produced CP4 EPSPS protein falls within the preset acceptance criterion (Table C-5), the MON 88302-produced CP4 EPSPS protein was considered to have equivalent functional activity to that of the *E. coli*-produced CP4 EPSPS protein.

Table C-5. CP4 EPSPS Functional Activity Assay

MON 88302-produced CP4 EPSPS Protein[1] (U/mg)	*E. coli*-produced CP4 EPSPS Protein[1] (U/mg)	Previously set acceptance limits[2] (U/mg)
4.93 ± 0.36	2.79 ± 0.26	1.40 – 5.58

[1]Value refers to mean and standard deviation calculated based on n = 6 which includes three replicate assays spectrophotometrically.
[2]Within 2-fold of the *E.coli*-produced CP4 EPSPS specific activity (2.79 ÷ 2 U/mg to 2.79 x 2 U/mg)

References for Appendix C

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Appendix D: Materials and Methods Used for the Analysis of the Levels of CP4 EPSPS Protein in MON 88302

D.1. Materials

Seed, forage, over-season leaf (OSL-1-4), and root (Root-1-2) tissue samples from MON 88302 were harvested from three field sites in the U.S. and three field sites in Canada during the 2009 growing season from starting seed lot 11225246. An *E. coli*-produced CP4 EPSPS protein (lot 10000739) was used as the analytical reference standard.

D.2. Characterization of the Materials

The identity of MON 88302 was confirmed by verifying the chain of custody documentation prior to analysis. To further confirm the identities of MON 88302 event-specific polymerase chain reaction (PCR) analyses were conducted on the harvested seed from each site. Any seed sample and its associated tissues, for which three or more pools out of four tested unexpectedly during PCR verification, were not analyzed in this study.

D.3. Field Design and Tissue Collection

Field trials were initiated during the 2009 planting season to generate MON 88302 samples at various canola growing locations in the U.S. and Canada. The forage, seed, OSL-1-4, and Root-1-2 tissue samples from the following field sites were analyzed: Power County, Idaho, U.S. (IDAF), Wilkin County, Minnesota, U.S (MNCA), McHenry County, North Dakota, U.S. (NDVA), Portage la Prairie, Manitoba, Canada (MBPL), Newton, Manitoba, Canada (MBNW) and Saskatoon, Saskatchewan, Canada (SKSA). These field sites were representative of canola producing regions suitable for commercial production. At each site, four replicated plots of plants containing MON 88302 were planted using a randomized complete block field design. OSL-1-4, forage, seed and Root-1-2 samples were collected from each replicated plot at all field sites. See Table V-1 for a detailed description of when the samples were collected.

From the IDAF site, seed and Root-2 samples were excluded from the study due to inclement weather during collection which impacted sample quality and quantity.

D.4. Tissue Processing and Protein Extraction

Tissue samples were shipped to Monsanto, St. Louis. The following tissues were not received by Sample Management: all OSL-2 tissue samples from sites MBPL, MBNW, and MNCA, and one Root-2 sample from site NDVA. The following tissues were received but not processed by sample management due to compromised sample integrity: all OSL-1 samples from site MBNW, one Root-1 sample from site SKSA, all Root-2 samples from site SKSA, and one Root-2 sample from site NDVA. The processed tissue samples were stored in a -80 °C freezer.

CP4 EPSPS protein was extracted from the tissue samples as described in Table D-1. CP4 EPSPS protein was extracted from all tissues samples using a Harbil Mixer with the appropriate amount of Tris-borate buffer with L-ascorbic acid (1× TBA) [0.1 M Tris, 0.1 M $Na_2B_4O_7 \bullet 10H_2O$, 0.005 M • $6H_2O$ $MgCl_2$, 0.05% (v/v) Tween-20 at pH 7.8, 0.2% (w/v) L-ascorbic acid]. Insoluble material was removed from all tissue extracts using a serum filter (Fisher Scientific, Pittsburgh, PA). The extracts were aliquotted and stored frozen in a -80 °C freezer until ELISA analysis.

Table D-1. Protein Extraction Methods for Tissue Samples

Sample Type	Tissue-to-Buffer Ratio	Extraction Buffer
Leaf[2]	1:100	1× TBA
Root[3]	1:100	1× TBA
Forage	1:100	1× TBA
Seed	1:100	1× TBA

[1]Over- season leaf (OSL-1, OSL-2, OSL-3, and OSL-4).
[2]Root (Root-1 and Root-2).

D.5. CP4 EPSPS Antibodies

Mouse monoclonal antibody clone 39B6.1 (IgG2a isotype, kappa light chain; lot 10002190) specific for the CP4 EPSPS protein was purified from mouse ascites fluid using Protein-A Sepharose affinity chromatography and was used as the capture antibody in the CP4 EPSPS ELISA. The concentration of the purified IgG was determined to be 2.3 mg/ml by spectrophotometric methods. Production of the 39B6.1 monoclonal antibody was performed by Strategic Biosolutions (Newark, DE). The purified antibody was stored in a buffer (pH 7.2) containing 20 mM sodium phosphate, 150 mM NaCl, and 15 ppm Proclin 300 (Sigma-Aldrich, St. Louis, MO).

The detection reagent was goat anti-CP4 EPSPS antibody, otherwise known as anti-protein 4 (Sigma-Aldrich, catalog number P-5867) conjugated to horseradish peroxidase (HRP).

D.6. CP4 EPSPS ELISA Method

Mouse anti-CP4 EPSPS antibodies were diluted in coating buffer (15 mM Na_2CO_3, 35 mM $NaHCO_3$, and 150 mM NaCl, pH 9.6) to a final concentration of 2.0 µg/ml, and immobilized onto 96-well microtiter plates followed by incubation in a 4 °C refrigerator for ≥8 hours. Prior to each step in the assay, plates were washed with 1× PBST. CP4 EPSPS protein standard or sample extract was added at 100 µl per well and incubated for 1 hour at 37 °C. The captured CP4 EPSPS protein was detected by the addition of 100 µl per well of anti-CP4 EPSPS HRP conjugate. Plates were developed by adding 100 µl per well of 3,3',5,5' tetramethyl-benzidine (TMB; Kirkegaard & Perry, Gaithersburg, MD). The enzymatic reaction was terminated by the addition of 100 µl per well of 6 M H_3PO_4. Quantification of the CP4 EPSPS protein was accomplished by interpolation from a CP4 EPSPS protein standard curve that ranged from 0.456-14.6 ng/ml.

D.7. Moisture Analysis

Tissue moisture content was determined using an IR-200 Moisture Analyzer (Denver Instrument Company, Arvada, CO). A homogeneous tissue-specific site pool (TSSP) was prepared consisting of samples of a given tissue type grown at a given site. The average percent moisture for each TSSP was calculated from triplicate analyses. A TSSP Dry Weight Conversion Factor (DWCF) was calculated as follows:

$$\text{DWCF} = 1 - \left(\frac{\text{Mean\% TSSP Moisture}}{100}\right)$$

The DWCF was used to convert protein levels assessed on a µg/g fresh weight (fw) basis into levels reported on a µg/g dry weight (dw) basis using the following calculation:

$$\text{Protein Level in Dry Weight} = \left(\frac{\text{Protein Level Fresh Weight}}{\text{DWCF}}\right)$$

The protein levels (ng/ml) that were reported to be less than or equal to the limit of detection (LOD) or less than the limit of quantitation (LOQ) on a fresh weight basis were not reported on a dry weight basis.

D.8. Data Analyses

All CP4 EPSPS ELISA plates were analyzed on a SPECTRAmax Plus 384 (Molecular Devices, Sunnyvale, CA) microplate spectrophotometer, using a dual wavelength detection method. All protein concentrations were determined by optical absorbance at a wavelength of 450 nm with a simultaneous reference reading of 620-650 nm. Data reduction analyses were performed using Molecular Devices SOFTmax PRO GxP version 5.0.1. Absorbance readings and protein standard concentrations were fitted with a four-parameter logistic curve. Following the interpolation from the standard curve, the amount of protein (ng/ml) in the tissue was converted to a µg/g fw basis for data that were greater than or equal to the LOQ. This conversion utilized a sample dilution factor and a tissue-to-buffer ratio. The protein values expressed as µg/g fw were also converted to µg/g dw by applying the DWCF. Microsoft Excel 2007 (Version (12.0.6535.5002) SP2 MSO (12.0.6535.5002) Microsoft, Redmond, WA) was used to calculate the CP4 EPSPS protein level in canola tissues. The sample means, standard deviations, and ranges were also calculated by Microsoft Excel 2007.

Any MON 88302 sample extracts that resulted in unexpectedly negative results by ELISA analysis were re-extracted twice for the protein of interest and re-analyzed by ELISA to confirm the results. Samples with confirmed unexpected results were omitted from all calculations.

Appendix E: Materials, Methods, and Individual-Site Results for Compositional Analysis of MON 88302 Canola Seed

E.1. Materials

Seed from MON 88302 (Seed Lot Number 11225246) and the conventional control (Seed Lot Number 11225244) was evaluated. The conventional control has background genetics similar to that of MON 88302 but does not contain the *cp4 epsps* expression cassette. The commercial reference varieties were seven conventional canola varieties (Table E-1).

Table E-1. Commercial Reference Canola Varieties

Material Name	Seed Lot Number	Field Sites[1]
Q2	10001931	MBPL, MBNW, SKSA, NDVA, MNCA
Hyola 401	10001850	NDVA, MBPL, SKSA
SP Armada	10001932	MBPL, SKSA, NDVA
Croplan 601	10001849	MBPL, SKSA, NDVA
SValof Sponsor	10002116	MNCA, MBNW
SValof Senator	10002115	MNCA, MBNW
DSV Ability	10002117	MNCA, MBNW

[1]Field sites described in Section E.3.

E.2. Characterization of the Materials

The identities of MON 88302, the conventional control, and commercial reference varieties were confirmed by verifying the chain of custody documentation prior to analysis. To further confirm the identities of MON 88302, the conventional control, and commercial reference varieties, event-specific polymerase chain reaction (PCR) analyses were conducted on the harvested seed from each site to confirm the presence or absence of the *cp4 epsps* expression cassette.

E.3. Field Production of the Samples

Seeds from the MON 88302, the conventional control and commercial reference varieties were collected from replicated plots at each of two U.S. sites [Wilkin County, Minnesota (MNCA); and McHenry County, North Dakota (NDVA)] and three Canadian sites [Portage la Prairie, Manitoba (MBPL); Newton, Manitoba (MBNW); and Saskatoon, Saskatchewan (SKSA)**].** Seeds were planted in a randomized complete block design with four replicates per site. The MON 88302 plots were treated with glyphosate applications between the 5-6 leaf stage, at a target rate of 1800 g a.e./ha. All samples at the field sites were grown under normal agronomic field conditions for their respective geographic regions. Seed samples were harvested from all plots and shipped at ambient temperature from the field sites to Monsanto Company (St. Louis, MO). Sub-samples were ground to a powder, stored in a freezer set to maintain -20 °C located at Monsanto Company (St.

Louis, MO), and then shipped on dry ice to Covance Laboratories Inc. (Madison, WI) for analysis.

E.4. Summary of Analytical Methods and Reference Standards

Ground grain samples were analyzed by Covance Laboratories Inc. Upon receipt, the samples were stored in a freezer set to maintain -20 °C until their use. Nutrients assessed in this analysis included proximates (ash, carbohydrates by calculation, moisture, protein, and fat), acid detergent fiber (ADF), neutral detergent fiber (NDF), total dietary fiber (TDF), amino acids, fatty acids (C8-C24), vitamin E (α-tocopherol) and minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc) in the grain. The toxicants assessed in grain included erucic acid and glucosinolates (alkyl glucosinolates, indolyl glucosinolates, and total glucosinolates). The anti-nutrients assessed in grain included phytic acid and sinapic acid.

E.4.1. Acid Detergent Fiber

The ANKOM2000 Fiber analyzer automated the process of removal of proteins, carbohydrates, and ash. Fats and pigments were removed with an acetone wash prior to analysis. The fibrous residue that is primarily cellulose, lignin, and insoluble protein complexes remained in the Ankom filter bag, and were determined gravimetrically. (Komarek et al., 1994; USDA, 1970). The results are reported on fresh weight basis. The limit of quantitation was 0.100%.

E.4.2. Amino Acid Composition

The following 18 amino acids were analyzed:

Total threonine	Total aspartic acid (including asparagine)
Total serine	Total tyrosine
Total phenylalanine	Total glutamic acid (including glutamine)
Total proline	Total histidine
Total glycine	Total lysine
Total alanine	Total arginine
Total valine	Total tryptophan
Total isoleucine	Total methionine
Total leucine	Total cystine (including cysteine)

The sample was assayed by three methods to obtain the full profile. Tryptophan required a base hydrolysis with sodium hydroxide. The sulfur-containing amino acids required an oxidation with performic acid prior to hydrolysis with hydrochloric acid. Analysis of the samples for the remaining amino acids was accomplished through direct acid hydrolysis with hydrochloric acid. Once hydrolyzed, the individual amino acids were then quantified using an automated amino acid analyzer (AOAC, 2005a). The limit of quantitation was 0.100 %.

Reference Standards:

- Thermo Scientific, K18 amino acid standard, H,, 2.5 ± 0.1 µmol/mL per constituent (except cystine 1.25 ± 0.1 µmol/mL), Lot Number KG137091
- Sigma, L-Tryptophan, 100%, Lot Number 097K0119
- Sigma/BioChemika, L-Cysteic Acid Monohydrate, 99.5% (used as 100%), Lot Number 1305674
- Sigma, L-Methionine Sulfone, 100%, Lot Number 047K1321
- Sigma, L-Norvaline, 100%, 087K1954

E.4.3. Ash

The sample was placed in an electric furnace at 550 °C and ignited. The nonvolatile matter remaining was quantified gravimetrically and calculated to determine percent ash (AOAC, 2005b). The limit of quantitation was 0.100%.

E.4.4. Carbohydrates

The total carbohydrate level was calculated by difference using the fresh weight-derived data and the following equation:

$$\% \text{ carbohydrates} = 100\% - (\% \text{ protein} + \% \text{ fat} + \% \text{ moisture} + \% \text{ ash})$$

The results are reported on fresh weight basis (USDA, 1973). The limit of quantitation was 0.100%.

E.4.5. Fat by Soxhlet Extraction

The sample was weighed into a cellulose thimble containing sodium sulfate and dried to remove excess moisture. Pentane was dripped through the sample to remove the fat. The extract was then evaporated, dried, and weighed (AOAC, 2005c). The results are reported on fresh weight basis. The limit of quantitation was 0.100%.

E.4.6. Fatty Acids as Triglycerides

The lipid was extracted, saponified with 0.5 N methanolic sodium hydroxide, and methylated with 14% boron trifluoride in methanol. The resulting methyl esters of the fatty acids were extracted with heptane containing an internal standard. The methyl esters of the fatty acids were analyzed by gas chromatography using external standards for quantitation (AOAC, 2005d; AOCS, 1997; 2007). The results are reported on fresh weight basis. The limit of quantitation was 0.0400%.

Reference Standards:

- Nu Chek Prep GLC Reference Standard Hazleton No. 1, *, Lot Number MA30-U
- Nu Chek Prep GLC Reference Standard Hazleton No. 2, *, Lot Number AU24-T
- Nu Chek Prep GLC Reference Standard Hazleton No. 3, *, Lot Number JY17-T
- Nu Chek Prep GLC Reference Standard Hazleton No. 4, *, Lot Number MA30-U
- Nu Chek Prep Methyl Gamma Linolenate, used as 100%, Lot Number U-63M-08-T
- Nu Chek Prep Methyl Tridecanoate, used as 100%, Lot Number N-13M-MA25-T
- Nu Chek Prep Methyl Erucate, used as 100%, Lot Numbers U-79M-JA28-T
- Nu Chek Prep Methyl Lignocerate, used as 100%, Lot Number N-24M-S8-T
- Nu Chek Prep Methyl Docosapentaenoate, used as 100%, Lot Number U-101M-D4-T
- Nu Chek Prep Methyl Docosahexaenoate, used as 100%, Lot Number U-84M-JA15-U

- Nu Chek Prep Methyl Eicosapentaenoate, used as 100%, Lot Number U-99M-S22-T
- Nu Chek Prep Methyl Nervonate, used as 100%, Lot Number U-88M-MA31-U
- Cayman Chemicals Stearidonic Acid Methyl Ester, 100%, Lot Number 0407775

*Overall purity of the sum of the mixture of components was used as 100%

E.4.7. Glucosinolates

Glucosinolates were extracted using 70% methanol at 75 °C. They were then purified and enzymatically desulfatated on ion-exchange resin. Determination was by reversed-phase high performance liquid chromatography with gradient elution and ultraviolet detection using an internal standard. Quantification was performed based on the relative responses to the internal standards. Peak identification was made based on retention times determined by comparing the chromatograms of internal standard(s) and three BCR certified oilseed rape controls (ISO, 1992). The results are reported on fresh weight basis. The limit of quantitation was 0.00300 µmole/g.

Reference Standard:

- Chromadex, Glucotropaeolin Potassium Salt, 98.7%, Lot Number 07300-304

E.4.8. ICP Emission Spectrometry

The sample was dried, precharred, and ashed overnight in a muffle furnace set to maintain 500 °C. The ashed sample was re-ashed with nitric acid, treated with hydrochloric acid, taken to dryness, and put into a solution of 5% hydrochloric acid. The amount of each element was determined at appropriate wavelengths by comparing the emission of the unknown sample, measured on the inductively coupled plasma spectrometer, with the emission of the standard solutions (AOAC, 2005e). The results are reported on fresh weight basis.

Reference Standards:

Inorganic Ventures Reference Standards and Limits of Quantitation:

Mineral	Lot Numbers	Concentration (µg/mL)	Limit of Quantitation (ppm)
Calcium	D2-MEB322092MCA, D2-MEB322094	200, 1000	20.0
Copper	D2-MEB322092MCA, D2-MEB322093MCA	2.00, 10.0	0.500
Iron	D2-MEB322092MCA, D2-MEB322095	10.0, 50.0	2.00
Magnesium	D2-MEB322092MCA, D2-MEB322093MCA	50.0, 250	20.0
Manganese	D2-MEB322092MCA, D2-MEB322093MCA	2.00, 10.0	0.300
Phosphorus	D2-MEB322092MCA, D2MEB322094	200, 1000	20.0
Potassium	D2-MEB322092MCA, D2-MEB322094	200, 1000	100
Sodium	D2-MEB322092MCA, D2-MEB322094	200, 1000	100
Zinc	D2-MEB322092MCA, D2-MEB322093MCA	10.0, 50.0	0.400

E.4.9. Moisture

The sample was dried in a vacuum oven at approximately 100 °C to a constant weight. The moisture weight loss was determined and converted to percent moisture (AOAC, 2005f). The results are reported on fresh weight basis. The limit of quantitation was 0.100%.

E.4.10. Neutral Detergent Fiber, Enzyme Method

The ANKOM2000 Fiber Analyzer automated the process of the removal of proteins, carbohydrates, and ash. The fats and pigments were removed with an acetone wash prior to analysis. Hemicellulose, cellulose, lignin and insoluble protein fraction was left in the filter bag and determined gravimetrically (AACC, 1998; Komarek et al., 1994; USDA, 1970). The results are reported on fresh weight basis. The limit of quantitation was 0.100%.

E.4.11. Phytic Acid

The sample was extracted using 0.5 M HCl with ultrasonication. Purification and concentration were accomplished on a silica-based anion-exchange column. The sample was analyzed on a polymer high-performance liquid chromatography column PRP-1, 5 µm (150 x 4.1mm) with a refractive index detector (Lehrfeld, 1989; Lehrfeld, 1994). The results are reported on fresh weight basis. The limit of quantitation was 0.100%.

Reference Standard:

Sigma-Aldrich, Phytic Acid Sodium Salt Hydrate, 96%, Lot Number 089K0159

E.4.12. Protein

The protein and other organic nitrogen in the sample were converted to ammonia by digesting the sample with sulfuric acid containing a catalyst mixture. The acid digest was made alkaline. The ammonia was distilled and then titrated with a previously standardized acid. The percent nitrogen was calculated and converted to equivalent protein using the factor 6.25 (AOAC, 2005g; AOCS, 1998). The results are reported on fresh weight basis. The limit of quantitation was 0.100%.

E.4.13. Sinapic Acid

The ground sample was extracted with methanol followed by alkaline hydrolysis and buffering prior to injection on an analytical high-performance liquid chromatography (HPLC) system for quantification of sinapic acid by ultra violet (UV) detection (Hagerman and Nicholson, 1982). The results are reported on fresh weight basis. The limit of quantitation was 200 ppm.

Reference Standard:

Sigma, Sinapic Acid, 99.3%, Lot No. 079K1171.

E.4.14. Total Dietary Fiber

Duplicate samples were gelatinized with α-amylase and digested with enzymes to break down starch and protein. Ethanol was added to each sample to precipitate the soluble fiber. The sample was filtered, and the residue was rinsed with ethanol and acetone to remove starch and protein degradation products and moisture. Protein content was determined for one of the duplicates; ash content was determined for the other. The total dietary fiber in the sample was calculated using protein and ash values (AOAC, 2005h). The results were reported on fresh weight basis. The limit of quantitation was 1.00%.

E.4.15. Vitamin E

The sample was saponified to break down any fat and release vitamin E. The saponified mixture was extracted with ethyl ether and then quantified by high-performance liquid chromatography using a silica column (Cort et al., 1983; McMurray et al., 1980; Speek et al., 1985). The results are reported on fresh weight basis. The limit of quantitation was 0.500 mg/100g.

Reference Standard:

USP, α-Tocopherol, 98.9%, Lot Number N0F068

E.5. Data Processing and Statistical Analysis

After compositional analyses were performed, data spreadsheets containing individual values for each analysis were sent to Monsanto Company for review. Data were then transferred to Certus International (Chesterfield, MO) where they were converted into the appropriate units and statistically analyzed. The formulas that were used for re-expression of composition data for statistical analysis are listed in Table E-2.

Table E-2. Re-expression Formulas for Statistical Analysis of Composition Data

Component	From (X)	To	Formula[1]
Proximates (excluding Moisture), Fiber, Phytic Acid	% fw	% dw	X/d
Alkyl Glucosinolate, Indolyl Glucosinolate, Total Glucosinolate	µmole/g fw	µmole/g dw	X/d
Sinapic Acid	ppm fw	% dw	$X/(10^4 d)$
Calcium, Magnesium, Phosphorus, Potassium, Sodium	ppm fw	g/100g dw	$X/(10^4 d)$
Copper, Iron, Manganese, Zinc	ppm fw	mg/kg dw	X/d
Vitamin E	mg/100g fw	mg/100g dw	X/d
Amino Acids (AA)	mg/g fw	% dw	X/(10d)
Fatty Acids (FA)	% fw	% Total FA	$(100)X_j/\Sigma X$, for each FA_j where ΣX is over all the FA

[1] 'X' is the individual sample value; 'd' is the fraction of the sample that is dry matter.

In order to complete a statistical analysis for a compositional component in this study, at least 50% of the values for a component had to be greater than the assay limit of quantitation (LOQ). Components with more than 50% of observations below the assay LOQ were excluded from summaries and analysis. The following 19 components with more than 50% of the observations below the assay LOQ were excluded: 8:0 caprylic acid, 10:0 capric acid, 12:0 lauric acid, 14:0 myristic acid, 14:1 myristoleic acid, 15:0 pentadecanoic acid, 15:1 pentadecenoic acid, 17:0 heptadecanoic acid, 17:1 heptadecenoic acid, 18:3 gamma-linolenic acid, 18:4 octadecatetraenoic acid; 20:2 eicosadienoic acid, 20:3 eicosatrienoic acid, 20:4 arachidonic acid, 20:5 eicosapentaenoic acid, 22:1 erucic acid, 22:5 docosapentaenoic acid, 22:6 docosahexaenoic acid, and sodium.

If less than 50% of the observations for a component were below the LOQ, individual analyses that were below the LOQ were assigned a value equal to one-half the LOQ. In this study 24 values for 24:0 lignoceric acid and 34 values for 24:1 nervonic acid were assigned a value of 0.02% total fw.

The data were assessed for potential outliers using a studentized PRESS (Predicted Residual Sum of Squares) calculation. A PRESS residual is the difference between any value and its value predicted from a statistical model that excludes the data point. The studentized version scales these residuals so that the values tend to have a standard normal distribution when outliers are absent. Thus, most values are expected to be between ± 3. Extreme data points that are also outside of the ± 6 studentized PRESS residual range are considered for exclusion, as outliers, from the final analyses. One 18:3 linolenic value from MON 88302 at the MNCA site, one alkyl glucosinolate value and one total glucosinolate value from one commercial reference at the MBPL site were identified as outliers, but the values were either similar to other nearby data points or were not the extreme highest or lowest value, and were not removed from statistical analysis. One carbohydrate value and one total fat value from one commercial reference at the MBNW site were extreme data points that were outside the ± 6 studentized PRESS residual range and were removed from the statistical analysis.

All canola components were statistically analyzed using a mixed model analysis of variance. The five replicated field sites were analyzed individually and as a combined data set. Individual replicated site analyses used model (1).

(1) $Y_{ij} = U + T_i + B_j + e_{ij}$,

where Y_{ij} = unique individual observation, U = overall mean, T_i = substance effect, B_j = random block effect, and e_{ij} = residual error.

Combined-site analyses used model (2).

(2) $Y_{ijk} = U + T_i + L_j + B(L)_{jk} + LT_{ij} + e_{ijk}$,

where Y_{ijk} = unique individual observation, U = overall mean, T_i = substance effect, L_j = random site effect, $B(L)_{jk}$ = random block within site effect, LT_{ij} = random site by substance interaction effect, and e_{ijk} = residual error.

For each compositional component, a range of observed values and a 99% tolerance interval were calculated. A tolerance interval is an interval that one can claim, with a specified degree of confidence, contains at least a specified proportion, p, of an entire sampled population for the parameter measured. The calculated tolerance intervals are expected to contain, with 95% confidence, 99% of the quantities expressed in the population of conventional canola. Each tolerance interval estimate was based upon the average observation for each unique reference material. Because negative quantities are not possible, negative calculated lower tolerance bounds were set to zero.

SAS (Version 9) software was used to generate all summary statistics and perform all analyses.

Report tables present p-values from SAS as either <0.001 or the actual value truncated to three decimal places.

Table E-3. Statistical Summary of Site MBNW Canola Seed Nutrient Content for MON 88302 vs. the Conventional Control

Analytical Component (Units)[1]	MON 88302[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88302 minus Control) Mean (S.E.) (Range)	95% Confidence Interval[3]	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Proximate (% dw)						
Ash	3.98 (0.087) (3.72 - 4.10)	3.84 (0.10) (3.66 - 4.03)	0.14 (0.13) (0.076 - 0.41)	-0.28, 0.56	0.367	3.32, 4.66 (2.98 - 4.52)
Carbohydrates	27.18 (0.29) (26.75 - 28.02)	26.02 (0.33) (25.81 - 26.35)	1.16 (0.40) (1.08 - 1.67)	-0.13, 2.45	0.063	23.12, 30.77 (22.53 - 29.96)
Moisture (% fw)	5.26 (0.16) (4.99 - 5.56)	4.90 (0.18) (4.69 - 5.13)	0.36 (0.24) (0.12 - 0.87)	-0.39, 1.12	0.225	4.33, 6.91 (4.09 - 8.48)
Protein	21.00 (0.62) (19.68 - 22.64)	20.78 (0.71) (20.29 - 21.61)	0.22 (0.94) (-0.86 - 0.49)	-2.78, 3.22	0.830	17.20, 30.08 (18.68 - 28.32)
Total Fat	47.84 (0.47) (46.87 - 49.26)	49.35 (0.54) (48.89 - 49.93)	-1.51 (0.72) (-2.28 - -0.61)	-3.79, 0.77	0.125	39.65, 51.24 (40.71 - 50.26)
Fiber (% dw)						
Acid Detergent Fiber	16.26 (0.63) (15.05 - 17.66)	14.93 (0.71) (13.64 - 16.34)	1.32 (0.81) (0.019 - 3.09)	-1.25, 3.90	0.199	6.95, 23.92 (9.75 - 21.22)

Table E -3. Statistical Summary of Site MBNW Canola Seed Nutrient Content for MON 88302 vs. the Conventional Control (continued)

Analytical Component (Units)[1]	MON 88302[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88302 minus Control) Mean (S.E.) (Range)	95% Confidence Interval[3]	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fiber (% dw)						
Neutral Detergent Fiber	19.08 (0.75) (17.16 - 21.36)	17.16 (0.87) (16.68 - 17.45)	1.92 (1.07) (-0.29 - 3.97)	-1.50, 5.33	0.171	10.07, 25.94 (10.93 - 22.75)
Total Dietary Fiber	22.93 (1.65) (19.17 - 27.81)	19.30 (1.87) (15.21 - 22.29)	3.63 (2.10) (-0.29 - 7.36)	-3.05, 10.32	0.181	13.97, 24.85 (12.64 - 26.47)
Amino Acid (% dw)						
Alanine	0.95 (0.026) (0.88 - 1.02)	0.95 (0.030) (0.93 - 0.98)	0.0018 (0.040) (-0.047 - 0.011)	-0.13, 0.13	0.967	0.77, 1.34 (0.87 - 1.27)
Arginine	1.35 (0.042) (1.23 - 1.44)	1.37 (0.049) (1.36 - 1.38)	-0.019 (0.065) (-0.12 - 0.025)	-0.23, 0.19	0.784	1.10, 1.93 (1.23 - 1.96)
Aspartic Acid	1.60 (0.057) (1.44 - 1.72)	1.58 (0.066) (1.55 - 1.64)	0.018 (0.087) (-0.10 - 0.090)	-0.26, 0.30	0.846	1.33, 2.12 (1.42 - 2.23)
Cystine	0.52 (0.020) (0.48 - 0.59)	0.51 (0.023) (0.50 - 0.54)	0.0048 (0.031) (-0.043 - 0.0090)	-0.093, 0.10	0.886	0.38, 0.83 (0.45 - 0.79)

Table E-3. Statistical Summary of Site MBNW Canola Seed Nutrient Content for MON 88302 vs. the Conventional Control (continued)

Analytical Component (Units)[1]	MON 88302[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88302 minus Control) Mean (S.E.) (Range)	95% Confidence Interval[3]	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dw)						
Glutamic Acid	3.68 (0.12) (3.37 - 4.02)	3.71 (0.14) (3.64 - 3.84)	-0.030 (0.19) (-0.27 - 0.043)	-0.64, 0.58	0.886	2.73, 5.89 (3.26 - 5.43)
Glycine	1.09 (0.028) (1.02 - 1.16)	1.09 (0.032) (1.06 - 1.12)	0.0020 (0.043) (-0.046 - 0.014)	-0.13, 0.14	0.965	0.96, 1.47 (1.01 - 1.50)
Histidine	0.59 (0.017) (0.55 - 0.64)	0.58 (0.019) (0.57 - 0.60)	0.0052 (0.026) (-0.023 - 0.0092)	-0.076, 0.087	0.851	0.47, 0.86 (0.54 - 0.80)
Isoleucine	0.87 (0.028) (0.81 - 0.94)	0.86 (0.032) (0.82 - 0.90)	0.010 (0.042) (-0.029 - 0.0069)	-0.12, 0.14	0.820	0.70, 1.22 (0.78 - 1.15)
Leucine	1.51 (0.044) (1.40 - 1.62)	1.51 (0.051) (1.48 - 1.56)	0.00056 (0.067) (-0.082 - 0.026)	-0.21, 0.21	0.993	1.21, 2.18 (1.36 - 2.07)
Lysine	1.31 (0.034) (1.22 - 1.41)	1.28 (0.040) (1.25 - 1.32)	0.033 (0.052) (-0.030 - 0.057)	-0.13, 0.20	0.573	1.02, 1.90 (1.20 - 1.68)

Table E-3. Statistical Summary of Site MBNW Canola Seed Nutrient Content for MON 88302 vs. the Conventional Control (continued)

Analytical Component (Units)[1]	MON 88302 Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88302 minus Control) Mean (S.E.) (Range)	95% Confidence Interval[3]	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dw)						
Methionine	0.41 (0.012) (0.40 - 0.45)	0.42 (0.014) (0.40 - 0.44)	-0.0029 (0.019) (-0.038 - 0.0034)	-0.063, 0.057	0.887	0.30, 0.65 (0.36 - 0.57)
Phenylalanine	0.91 (0.025) (0.84 - 0.97)	0.91 (0.029) (0.90 - 0.93)	0.0043 (0.038) (-0.053 - 0.019)	-0.12, 0.12	0.916	0.77, 1.26 (0.84 - 1.25)
Proline	1.27 (0.038) (1.20 - 1.35)	1.24 (0.044) (1.20 - 1.29)	0.029 (0.058) (-0.030 - 0.036)	-0.16, 0.21	0.659	0.90, 2.01 (1.12 - 1.78)
Serine	0.96 (0.029) (0.87 - 1.03)	0.95 (0.033) (0.94 - 0.97)	0.0032 (0.044) (-0.077 - 0.051)	-0.14, 0.14	0.945	0.81, 1.32 (0.88 - 1.30)
Threonine	0.94 (0.022) (0.88 - 0.98)	0.94 (0.025) (0.92 - 0.96)	0.0053 (0.034) (-0.044 - 0.058)	-0.10, 0.11	0.884	0.82, 1.20 (0.84 - 1.22)
Tryptophan	0.21 (0.017) (0.17 - 0.26)	0.21 (0.020) (0.19 - 0.25)	-0.0040 (0.026) (-0.037 - - 0.0025)	-0.088, 0.080	0.889	0.13, 0.35 (0.17 - 0.32)

Table E-3. Statistical Summary of Site MBNW Canola Seed Nutrient Content for MON 88302 vs. the Conventional Control (continued)

Analytical Component (Units)[1]	MON 88302[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88302 minus Control) Mean (S.E.) (Range)	95% Confidence Interval[3]	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dw)						
Tyrosine	0.64 (0.015) (0.59 - 0.66)	0.63 (0.017) (0.63 - 0.64)	0.0046 (0.022) (-0.037 - 0.016)	-0.066, 0.075	0.850	0.57, 0.81 (0.60 - 0.84)
Valine	1.12 (0.036) (1.04 - 1.21)	1.10 (0.041) (1.05 - 1.15)	0.021 (0.054) (-0.032 - 0.014)	-0.15, 0.19	0.719	0.92, 1.55 (1.01 - 1.46)
Fatty Acid (% Total FA)						
16:0 Palmitic	4.10 (0.029) (4.02 - 4.16)	4.00 (0.033) (3.97 - 4.06)	0.10 (0.044) (0.0047 - 0.18)	-0.039, 0.24	0.105	2.84, 5.26 (3.55 - 4.69)
16:1 Palmitoleic	0.21 (0.0031) (0.20 - 0.21)	0.23 (0.0036) (0.22 - 0.23)	-0.022 (0.0044) (-0.028 - -0.015)	-0.036, -0.0081	0.015	0.17, 0.30 (0.19 - 0.27)
18:0 Stearic	1.73 (0.039) (1.64 - 1.87)	1.97 (0.045) (1.93 - 2.01)	-0.24 (0.060) (-0.35 - -0.059)	-0.43, -0.049	0.028	0.90, 3.05 (1.50 - 2.64)
18:1 Oleic	63.40 (0.19) (62.94 - 64.03)	65.71 (0.22) (65.55 - 65.93)	-2.30 (0.29) (-3.00 - -1.52)	-3.24, -1.37	0.004	56.13, 70.69 (57.86 - 68.53)

Table E-3. Statistical Summary of Site MBNW Canola Seed Nutrient Content for MON 88302 vs. the Conventional Control (continued)

Analytical Component (Units)[1]	MON 88302[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88302 minus Control) Mean (S.E.) (Range)	95% Confidence Interval[3]	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fatty Acid (% Total FA)						
18:2 Linoleic	19.27 (0.16) (18.82 - 19.66)	17.89 (0.19) (17.70 - 18.17)	1.38 (0.25) (0.65 - 1.96)	0.59, 2.17	0.011	12.60, 24.49 (14.12 - 22.57)
18:3 Linolenic	9.19 (0.091) (8.88 - 9.42)	8.12 (0.10) (7.98 - 8.25)	1.08 (0.14) (0.76 - 1.43)	0.64, 1.52	0.004	6.96, 11.73 (7.99 - 10.94)
20:0 Arachidic	0.52 (0.010) (0.50 - 0.54)	0.56 (0.012) (0.54 - 0.58)	-0.042 (0.015) (-0.081 - -0.0032)	-0.091, 0.0064	0.069	0.45, 0.80 (0.53 - 0.71)
20:1 Eicosenoic	1.08 (0.021) (1.06 - 1.15)	1.03 (0.024) (1.00 - 1.08)	0.055 (0.032) (-0.016 - 0.064)	-0.046, 0.16	0.180	0.83, 1.68 (1.04 - 1.56)
22:0 Behenic	0.25 (0.0058) (0.24 - 0.26)	0.26 (0.0067) (0.24 - 0.27)	-0.010 (0.0089) (-0.030 - 0.00017)	-0.038, 0.018	0.337	0.19, 0.43 (0.27 - 0.38)
24:0 Lignoceric	0.15 (0.0053) (0.14 - 0.16)	0.15 (0.0061) (0.15 - 0.15)	0.0020 (0.0081) (-0.0041 - 0.0063)	-0.024, 0.028	0.824	0.033, 0.25 (0.044 - 0.21)

Table E-3. Statistical Summary of Site MBNW Canola Seed Nutrient Content for MON 88302 vs. the Conventional Control (continued)

Analytical Component (Units)[1]	MON 88302[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88302 minus Control) Mean (S.E.) (Range)	95% Confidence Interval[3]	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fatty Acid (% Total FA)						
24:1 Nervonic	0.096 (0.027) (0.046 - 0.12)	0.090 (0.031) (0.046 - 0.12)	0.0065 (0.041) (-0.069 - 0.072)	-0.13, 0.14	0.884	0.041, 0.18 (0.044 - 0.20)
Mineral						
Calcium (g/100g dw)	0.48 (0.010) (0.45 - 0.51)	0.44 (0.012) (0.43 - 0.46)	0.038 (0.016) (0.017 - 0.081)	-0.012, 0.089	0.095	0.16, 0.61 (0.25 - 0.53)
Copper (mg/kg dw)	3.72 (0.040) (3.61 - 3.83)	3.41 (0.046) (3.36 - 3.44)	0.32 (0.061) (0.22 - 0.40)	0.12, 0.51	0.013	2.00, 4.43 (2.52 - 4.93)
Iron (mg/kg dw)	42.22 (1.46) (40.55 - 43.60)	46.51 (1.66) (41.65 - 51.30)	-4.30 (1.90) (-8.99 - -1.10)	-10.33, 1.74	0.108	23.39, 86.23 (39.16 - 77.92)
Magnesium (g/100g dw)	0.34 (0.010) (0.31 - 0.35)	0.33 (0.012) (0.31 - 0.35)	0.0078 (0.016) (0.0036 - 0.039)	-0.042, 0.057	0.651	0.32, 0.43 (0.30 - 0.45)
Manganese (mg/kg dw)	39.62 (1.70) (35.28 - 43.84)	39.54 (1.96) (37.35 - 41.11)	0.078 (2.60) (-3.95 - 6.49)	-8.18, 8.34	0.977	14.85, 61.05 (25.00 - 54.11)

Table E-3. Statistical Summary of Site MBNW Canola Seed Nutrient Content for MON 88302 vs the Conventional Control (continued)

Analytical Component (Units)[1]	MON 88302[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88302 minus Control) Mean (S.E.) (Range)	95% Confidence Interval[3]	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Mineral						
Phosphorus (g/100g dw)	0.72 (0.041) (0.60 - 0.78)	0.72 (0.047) (0.61 - 0.79)	0.0064 (0.062) (-0.034 - 0.16)	-0.19, 0.20	0.925	0.38, 1.06 (0.44 - 0.87)
Potassium (g/100g dw)	0.56 (0.0096) (0.54 - 0.57)	0.56 (0.011) (0.54 - 0.58)	-0.0054 (0.015) (-0.0098 - 0.021)	-0.052, 0.041	0.734	0.39, 0.96 (0.50 - 0.92)
Zinc (mg/kg dw)	34.91 (1.09) (32.40 - 37.15)	30.24 (1.26) (28.46 - 32.84)	4.66 (1.66) (-0.44 - 7.72)	-0.63, 9.95	0.067	20.19, 48.23 (22.18 - 47.61)
Vitamin (mg/100g dw)						
Vitamin E (α-tocopherol)	13.06 (0.31) (12.22 - 13.47)	9.36 (0.36) (8.89 - 10.15)	3.70 (0.48) (3.07 - 4.46)	2.17, 5.23	0.004	3.88, 17.28 (2.62 - 14.84)

[1]dw = dry weight; fw = fresh weight; FA = fatty acid.
[2] MON 88302 treated with glyphosate.
[3]Mean (S.E.) = least-square mean (standard error); CI = confidence interval.
[4]Control refers to the genetically similar, conventional control.
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial conventional references. Negative limits were set to zero.

Table E-4. Statistical Summary of Site MBNW Canola Seed Anti-nutrient Content for MON 88302 vs. the Conventional Control

Analytical Component (Units)[1]	MON 88302[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88302 minus Control) Mean (S.E.) (Range)	95% Confidence Interval[3]	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Anti-nutrient						
Alkyl Glucosinolate (µmole/g dw)	5.19 (0.28) (4.47 - 5.87)	5.43 (0.31) (4.85 - 6.16)	-0.23 (0.28) (-0.38 - 0.091)	-1.13, 0.67	0.472	0, 29.02 (2.32 - 28.33)
Indolyl Glucosinolate (µmole/g dw)	4.23 (0.57) (2.92 - 5.75)	4.01 (0.65) (2.92 - 5.31)	0.22 (0.86) (-1.32 - 2.83)	-2.53, 2.97	0.817	1.37, 6.62 (1.84 - 7.18)
Phytic Acid (% dw)	2.06 (0.17) (1.73 - 2.46)	2.27 (0.20) (1.77 - 2.56)	-0.21 (0.26) (-0.67 - 0.68)	-1.05, 0.63	0.489	0.70, 3.52 (1.10 - 2.71)
Sinapic Acid (% dw)	1.02 (0.014) (0.99 - 1.06)	0.92 (0.014) (0.92 - 0.94)	0.095 (0.0093) (0.076 - 0.11)	0.066, 0.12	0.001	0.57, 1.13 (0.48 - 0.99)
Total Glucosinolate (µmole/g dw)	9.60 (0.77) (7.60 - 11.42)	9.61 (0.88) (8.44 - 11.56)	-0.014 (1.05) (-1.54 - 2.98)	-3.35, 3.32	0.990	0, 32.20 (5.52 - 31.98)

[1]dw = dry weight.
[2] MON 88302 treated with glyphosate
[3]Mean (S.E.) = least-square mean (standard error); CI = confidence interval.
[4]Control refers to the genetically similar, conventional control.
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial conventional references. Negative limits were set to zero.

Table E-5. Statistical Summary of Site MBPL Canola Seed Nutrient Content for MON 88302 vs. the Conventional Control

Analytical Component (Units)[1]	MON 88302[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88302 minus Control) Mean (S.E.) (Range)	95% Confidence Interval[3]	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Proximate (% dw)						
Ash	4.15 (0.070) (3.99 - 4.38)	4.28 (0.080) (4.17 - 4.38)	-0.14 (0.089) (-0.21 - 0.020)	-0.38, 0.11	0.201	3.32, 4.66 (2.98 - 4.52)
Carbohydrates	27.51 (0.48) (26.55 - 28.81)	28.11 (0.56) (26.87 - 28.73)	-0.59 (0.74) (-2.18 - 1.94)	-2.64, 1.45	0.466	23.12, 30.77 (22.53 - 29.96)
Moisture (% fw)	5.68 (0.12) (5.45 - 5.93)	5.24 (0.14) (4.93 - 5.47)	0.44 (0.19) (0.12 - 0.67)	-0.078, 0.95	0.077	4.33, 6.91 (4.09 - 8.48)
Protein	23.70 (0.26) (23.17 - 24.33)	23.46 (0.30) (23.03 - 24.12)	0.23 (0.40) (-0.95 - 1.30)	-0.87, 1.33	0.590	17.20, 30.08 (18.68 - 28.32)
Total Fat	44.66 (0.36) (43.96 - 45.72)	44.20 (0.41) (43.65 - 44.85)	0.46 (0.49) (-0.84 - 1.11)	-0.91, 1.83	0.405	39.65, 51.24 (40.71 - 50.26)
Fiber (% dw)						
Acid Detergent Fiber	16.75 (0.70) (15.17 - 18.19)	14.19 (0.73) (12.59 - 16.16)	2.55 (0.46) (2.03 - 3.57)	1.28, 3.83	0.005	6.95, 23.92 (9.75 - 21.22)

Table E-5. Statistical Summary of Site MBPL Canola Seed Nutrient Content for MON 88302 vs. the Conventional Control (continued)

Analytical Component (Units)[1]	MON 88302[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88302 minus Control) Mean (S.E.) (Range)	95% Confidence Interval[3]	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fiber (% dw)						
Neutral Detergent Fiber	19.45 (0.50) (18.35 - 20.02)	16.87 (0.57) (15.44 - 18.06)	2.58 (0.66) (1.50 - 4.43)	0.74, 4.43	0.017	10.07, 25.94 (10.93 - 22.75)
Total Dietary Fiber	22.61 (1.30) (18.67 - 24.98)	17.91 (1.50) (14.58 - 20.42)	4.70 (1.99) (3.52 - 9.96)	-0.82, 10.23	0.077	13.97, 24.85 (12.64 - 26.47)
Amino Acid (% dw)						
Alanine	1.06 (0.013) (1.04 - 1.08)	1.04 (0.015) (1.02 - 1.09)	0.017 (0.020) (-0.049 - 0.059)	-0.040, 0.073	0.460	0.77, 1.34 (0.87 - 1.27)
Arginine	1.57 (0.033) (1.51 - 1.64)	1.54 (0.039) (1.48 - 1.65)	0.031 (0.051) (-0.14 - 0.15)	-0.11, 0.17	0.578	1.10, 1.93 (1.23 - 1.96)
Aspartic Acid	1.84 (0.024) (1.81 - 1.89)	1.79 (0.027) (1.73 - 1.85)	0.047 (0.029) (-0.023 - 0.086)	-0.034, 0.13	0.179	1.33, 2.12 (1.42 - 2.23)
Cystine	0.55 (0.016) (0.50 - 0.59)	0.57 (0.019) (0.53 - 0.60)	-0.013 (0.025) (-0.054 - 0.052)	-0.082, 0.056	0.625	0.38, 0.83 (0.45 - 0.79)

Table E-5. Statistical Summary of Site MBPL Canola Seed Nutrient Content for MON 88302 vs. the Conventional Control (continued)

Analytical Component (Units)[1]	MON 88302[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88302 minus Control) Mean (S.E.) (Range)	95% Confidence Interval[3]	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dw)						
Glutamic Acid	4.26 (0.084) (4.15 - 4.41)	4.24 (0.097) (4.05 - 4.53)	0.017 (0.13) (-0.37 - 0.36)	-0.34, 0.37	0.903	2.73, 5.89 (3.26 - 5.43)
Glycine	1.23 (0.013) (1.21 - 1.24)	1.21 (0.015) (1.19 - 1.26)	0.013 (0.020) (-0.047 - 0.044)	-0.043, 0.070	0.542	0.96, 1.47 (1.01 - 1.50)
Histidine	0.65 (0.0098) (0.63 - 0.67)	0.65 (0.011) (0.62 - 0.68)	0.0039 (0.015) (-0.041 - 0.044)	-0.037, 0.045	0.806	0.47, 0.86 (0.54 - 0.80)
Isoleucine	0.99 (0.011) (0.95 - 1.01)	0.98 (0.012) (0.96 - 1.00)	0.0090 (0.016) (-0.050 - 0.034)	-0.036, 0.054	0.609	0.70, 1.22 (0.78 - 1.15)
Leucine	1.73 (0.021) (1.70 - 1.76)	1.71 (0.024) (1.66 - 1.78)	0.027 (0.032) (-0.076 - 0.086)	-0.063, 0.12	0.448	1.21, 2.18 (1.36 - 2.07)
Lysine	1.41 (0.019) (1.37 - 1.45)	1.40 (0.022) (1.36 - 1.45)	0.0064 (0.029) (-0.067 - 0.086)	-0.073, 0.086	0.833	1.02, 1.90 (1.20 - 1.68)

Table E-5. Statistical Summary of Site MBPL Canola Seed Nutrient Content for MON 88302 vs. the Conventional Control (continued)

Analytical Component (Units)[1]	MON 88302[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88302 minus Control) Mean (S.E.) (Range)	95% Confidence Interval[3]	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dw)						
Methionine	0.46 (0.0083) (0.43 - 0.48)	0.47 (0.0095) (0.45 - 0.49)	-0.0073 (0.013) (-0.024 - 0.030)	-0.042, 0.028	0.593	0.30, 0.65 (0.36 - 0.57)
Phenylalanine	1.05 (0.011) (1.04 - 1.05)	1.03 (0.013) (1.01 - 1.07)	0.014 (0.017) (-0.032 - 0.039)	-0.032, 0.060	0.443	0.77, 1.26 (0.84 - 1.25)
Proline	1.39 (0.033) (1.32 - 1.47)	1.36 (0.038) (1.30 - 1.45)	0.021 (0.050) (-0.12 - 0.17)	-0.12, 0.16	0.696	0.90, 2.01 (1.12 - 1.78)
Serine	1.08 (0.016) (1.05 - 1.09)	1.07 (0.018) (1.04 - 1.12)	0.012 (0.018) (-0.037 - 0.052)	-0.039, 0.063	0.559	0.81, 1.32 (0.88 - 1.30)
Threonine	1.06 (0.013) (1.04 - 1.06)	1.02 (0.015) (0.99 - 1.07)	0.031 (0.016) (-0.0054 - 0.065)	-0.013, 0.075	0.119	0.82, 1.20 (0.84 - 1.22)
Tryptophan	0.25 (0.0075) (0.24 - 0.25)	0.26 (0.0087) (0.25 - 0.27)	-0.011 (0.011) (-0.014 - -0.0041)	-0.043, 0.021	0.388	0.13, 0.35 (0.17 - 0.32)

Table E-5. Statistical Summary of Site MBPL Canola Seed Nutrient Content for MON 88302 vs. the Conventional Control (continued)

Analytical Component (Units)[1]	MON 88302[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88302 minus Control) Mean (S.E.) (Range)	95% Confidence Interval[3]	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dw)						
Tyrosine	0.72 (0.0065) (0.72 - 0.73)	0.71 (0.0069) (0.69 - 0.73)	0.017 (0.0052) (0.0078 - 0.028)	0.0030, 0.032	0.028	0.57, 0.81 (0.60 - 0.84)
Valine	1.26 (0.014) (1.21 - 1.29)	1.25 (0.016) (1.23 - 1.27)	0.016 (0.021) (-0.058 - 0.054)	-0.041, 0.074	0.474	0.92, 1.55 (1.01 - 1.46)
Fatty Acid (% Total FA)						
16:0 Palmitic	4.25 (0.031) (4.20 - 4.29)	4.34 (0.035) (4.23 - 4.41)	-0.090 (0.047) (-0.22 - 0.030)	-0.22, 0.040	0.126	2.84, 5.26 (3.55 - 4.69)
16:1 Palmitoleic	0.23 (0.0035) (0.22 - 0.23)	0.25 (0.0040) (0.24 - 0.26)	-0.026 (0.0053) (-0.031 - -0.015)	-0.040, -0.011	0.008	0.17, 0.30 (0.19 - 0.27)
18:0 Stearic	1.58 (0.023) (1.55 - 1.59)	1.87 (0.026) (1.79 - 1.93)	-0.29 (0.028) (-0.34 - -0.22)	-0.37, -0.22	<0.001	0.90, 3.05 (1.50 - 2.64)
18:1 Oleic	62.06 (0.11) (61.82 - 62.35)	64.30 (0.13) (64.19 - 64.56)	-2.24 (0.13) (-2.40 - -1.84)	-2.59, -1.88	<0.001	56.13, 70.69 (57.86 - 68.53)

Table E-5. Statistical Summary of Site MBPL Canola Seed Nutrient Content for MON 88302 vs. the Conventional Control (continued)

Analytical Component (Units)[1]	MON 88302[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88302 minus Control) Mean (S.E.) (Range)	95% Confidence Interval[3]	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fatty Acid (% Total FA)						
18:2 Linoleic	20.43 (0.088) (20.13 - 20.66)	19.18 (0.099) (19.01 - 19.26)	1.25 (0.11) (0.92 - 1.41)	0.95, 1.55	<0.001	12.60, 24.49 (14.12 - 22.57)
18:3 Linolenic	9.28 (0.085) (9.12 - 9.43)	7.74 (0.091) (7.52 - 7.92)	1.54 (0.073) (1.35 - 1.67)	1.34, 1.74	<0.001	6.96, 11.73 (7.99 - 10.94)
20:0 Arachidic	0.53 (0.0046) (0.52 - 0.54)	0.60 (0.0049) (0.59 - 0.61)	-0.070 (0.0042) (-0.079 - -0.063)	-0.082, -0.059	<0.001	0.45, 0.80 (0.53 - 0.71)
20:1 Eicosenoic	1.09 (0.0059) (1.08 - 1.10)	1.08 (0.0068) (1.06 - 1.09)	0.011 (0.0089) (-0.013 - 0.042)	-0.014, 0.035	0.298	0.83, 1.68 (1.04 - 1.56)
22:0 Behenic	0.27 (0.0027) (0.26 - 0.27)	0.30 (0.0032) (0.29 - 0.31)	-0.040 (0.0039) (-0.047 - -0.030)	-0.050, -0.029	<0.001	0.19, 0.43 (0.27 - 0.38)
24:0 Lignoceric	0.16 (0.0046) (0.16 - 0.17)	0.19 (0.0054) (0.18 - 0.19)	-0.023 (0.0068) (-0.024 - -0.022)	-0.042, -0.0038	0.029	0.033, 0.25 (0.044 - 0.21)

Table E-5. Statistical Summary of Site MBPL Canola Seed Nutrient content for MON 88302 vs. the Conventional Control (continued)

Analytical Component (Units)[1]	MON 88302[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88302 minus Control) Mean (S.E.) (Range)	95% Confidence Interval[3]	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fatty Acid (% Total FA)						
24:1 Nervonic	0.13 (0.0066) (0.12 - 0.13)	0.16 (0.0076) (0.15 - 0.17)	-0.033 (0.010) (-0.052 - -0.014)	-0.061, -0.0047	0.031	0.041, 0.18 (0.044 - 0.20)
Mineral						
Calcium (g/100g dw)	0.36 (0.0051) (0.35 - 0.37)	0.34 (0.0059) (0.32 - 0.34)	0.021 (0.0078) (0.0015 - 0.035)	-0.00057, 0.043	0.053	0.16, 0.61 (0.25 - 0.53)
Copper (mg/kg dw)	3.47 (0.082) (3.35 - 3.56)	3.97 (0.094) (3.68 - 4.18)	-0.50 (0.12) (-0.83 - -0.23)	-0.84, -0.15	0.016	2.00, 4.43 (2.52 - 4.93)
Iron (mg/kg dw)	44.13 (0.64) (42.80 - 45.09)	51.01 (0.73) (49.75 - 52.89)	-6.87 (0.90) (-9.20 - -4.80)	-9.38, -4.37	0.001	23.39, 86.23 (39.16 - 77.92)
Magnesium (g/100g dw)	0.41 (0.0070) (0.39 - 0.42)	0.41 (0.0081) (0.40 - 0.42)	-0.0029 (0.011) (-0.022 - 0.021)	-0.033, 0.027	0.797	0.32, 0.43 (0.30 - 0.45)
Manganese (mg/kg dw)	39.33 (0.90) (37.24 - 41.46)	37.78 (1.03) (36.29 - 39.99)	1.55 (1.37) (-1.82 - 4.38)	-2.25, 5.34	0.321	14.85, 61.05 (25.00 - 54.11)

Table E-5. Statistical Summary of Site MBPL Canola Seed Nutrient Content for MON 88302 vs. the Conventional Control (continued)

Analytical Component (Units)[1]	MON 88302[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88302 minus Control) Mean (S.E.) (Range)	95% Confidence Interval[3]	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Mineral						
Phosphorus (g/100g dw)	0.78 (0.0092) (0.75 - 0.80)	0.81 (0.011) (0.80 - 0.82)	-0.031 (0.014) (-0.033 - -0.0099)	-0.069, 0.0077	0.090	0.38, 1.06 (0.44 - 0.87)
Potassium (g/100g dw)	0.70 (0.025) (0.63 - 0.76)	0.77 (0.027) (0.77 - 0.81)	-0.068 (0.019) (-0.097 - -0.017)	-0.12, -0.015	0.023	0.39, 0.96 (0.50 - 0.92)
Zinc (mg/kg dw)	31.25 (0.49) (30.45 - 32.05)	33.88 (0.56) (32.82 - 35.76)	-2.63 (0.75) (-4.50 - -1.02)	-4.70, -0.56	0.024	20.19, 48.23 (22.18 - 47.61)
Vitamin (mg/100g dw)						
Vitamin E (α-tocopherol)	11.50 (0.24) (10.70 - 12.20)	7.63 (0.27) (7.50 - 7.72)	3.88 (0.36) (3.20 - 4.23)	2.87, 4.89	<0.001	3.88, 17.28 (2.62 - 14.84)

[1]dw = dry weight; fw = fresh weight; FA = fatty acid.
[2] MON 88302 treated with glyphosate.
[3]Mean (S.E.) = least-square mean (standard error); CI = confidence interval.
[4]Control refers to the genetically similar, conventional control.
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial conventional references. Negative limits were set to zero.

Table E-6. Statistical Summary of Site MBPL Canola Seed Anti-nutrient Content for MON 88302 vs. the Conventional Control

Analytical Component (Units)[1]	MON 88302[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88302 minus Control) Mean (S.E.) (Range)	95% Confidence Interval[3]	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Anti-nutrient						
Alkyl Glucosinolate (µmole/g dw)	2.98 (0.64) (1.91 - 4.03)	5.03 (0.74) (3.06 - 6.50)	-2.05 (0.98) (-2.68 - -1.15)	-4.76, 0.66	0.103	0, 29.02 (2.32 - 28.33)
Indolyl Glucosinolate (µmole/g dw)	3.90 (0.69) (1.67 - 5.76)	4.29 (0.79) (3.26 - 5.89)	-0.39 (0.93) (-1.59 - 0.96)	-2.98, 2.20	0.697	1.37, 6.62 (1.84 - 7.18)
Phytic Acid (% dw)	2.36 (0.066) (2.19 - 2.58)	2.39 (0.076) (2.35 - 2.41)	-0.027 (0.10) (-0.15 - 0.17)	-0.31, 0.25	0.803	0.70, 3.52 (1.10 - 2.71)
Sinapic Acid (% dw)	0.97 (0.0080) (0.95 - 0.99)	0.86 (0.0090) (0.86 - 0.86)	0.10 (0.010) (0.085 - 0.12)	0.076, 0.13	<0.001	0.57, 1.13 (0.48 - 0.99)
Total Glucosinolate (µmole/g dw)	7.01 (1.30) (3.66 - 9.77)	9.40 (1.49) (6.42 - 12.59)	-2.39 (1.83) (-2.82 - -0.44)	-7.45, 2.68	0.261	0, 32.20 (5.52 - 31.98)

[1]dw = dry weight; fw = fresh weight; FA = fatty acid.
[2] MON 88302 treated with glyphosate.
[3]Mean (S.E.) = least-square mean (standard error); CI = confidence interval.
[4]Control refers to the genetically similar, conventional control.
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial conventional references. Negative limits were set to zero

Table E-7. Statistical Summary of Site MNCA Canola Seed Nutrient Content for MON 88302 vs. the Conventional Control

Analytical Component (Units)[1]	MON 88302[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88302 minus Control) Mean (S.E.) (Range)	95% Confidence Interval[3]	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Proximate (% dw)						
Ash	4.35 (0.23) (4.20 - 4.45)	4.18 (0.20) (3.76 - 5.10)	0.17 (0.30) (0.33 - 0.64)	-0.60, 0.94	0.591	3.32, 4.66 (2.98 - 4.52)
Carbohydrates	27.31 (0.35) (26.27 - 27.90)	25.99 (0.30) (25.57 - 26.55)	1.32 (0.46) (-0.29 - 1.92)	0.13, 2.51	0.035	23.12, 30.77 (22.53 - 29.96)
Moisture (% fw)	5.52 (0.13) (5.37 - 5.61)	6.69 (0.11) (6.33 - 6.98)	-1.17 (0.17) (-1.53 - -0.72)	-1.60, -0.74	<0.001	4.33, 6.91 (4.09 - 8.48)
Protein	22.00 (0.70) (21.51 - 22.03)	23.23 (0.61) (21.50 - 24.27)	-1.23 (0.81) (-2.29 - 0.53)	-3.30, 0.85	0.189	17.20, 30.08 (18.68 - 28.32)
Total Fat	46.04 (0.72) (45.76 - 47.55)	46.59 (0.69) (45.26 - 48.05)	-0.55 (0.46) (-0.78 - -0.21)	-1.72, 0.62	0.280	39.65, 51.24 (40.71 - 50.26)
Fiber (% dw)						
Acid Detergent Fiber	17.89 (0.87) (15.99 - 20.24)	17.66 (0.75) (16.11 - 18.71)	0.23 (1.15) (-2.71 - 2.09)	-2.73, 3.20	0.847	6.95, 23.92 (9.75 - 21.22)

Table E-7. Statistical Summary of Site MNCA Canola Seed Nutrient Content for MON 88302 vs. the Conventional Control (continued)

Analytical Component (Units)[1]	MON 88302[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88302 minus Control) Mean (S.E.) (Range)	95% Confidence Interval[3]	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fiber (% dw)						
Neutral Detergent Fiber	19.55 (0.82) (17.90 - 21.19)	18.78 (0.71) (17.83 - 19.58)	0.76 (1.08) (-1.56 - 3.36)	-2.01, 3.54	0.511	10.07, 25.94 (10.93 - 22.75)
Total Dietary Fiber	20.18 (1.55) (16.91 - 22.24)	19.75 (1.35) (17.40 - 23.00)	0.44 (2.06) (-0.49 - 3.79)	-4.85, 5.72	0.839	13.97, 24.85 (12.64 - 26.47)
Amino Acid (% dw)						
Alanine	0.98 (0.027) (0.97 - 0.98)	1.05 (0.023) (0.98 - 1.10)	-0.070 (0.032) (-0.12 - -0.014)	-0.15, 0.014	0.084	0.77, 1.34 (0.87 - 1.27)
Arginine	1.40 (0.054) (1.38 - 1.39)	1.53 (0.048) (1.40 - 1.65)	-0.13 (0.063) (-0.27 - -0.010)	-0.29, 0.029	0.089	1.10, 1.93 (1.23 - 1.96)
Aspartic Acid	1.59 (0.067) (1.57 - 1.60)	1.79 (0.058) (1.61 - 1.97)	-0.20 (0.082) (-0.37 - -0.045)	-0.41, 0.0098	0.057	1.33, 2.12 (1.42 - 2.23)
Cystine	0.57 (0.025) (0.53 - 0.58)	0.55 (0.022) (0.52 - 0.61)	0.015 (0.031) (0.012 - 0.035)	-0.064, 0.094	0.650	0.38, 0.83 (0.45 - 0.79)

Table E-7. Statistical Summary of Site MNCA Canola Seed Nutrient Content for MON 88302 vs. the Conventional Control (continued)

Analytical Component (Units)[1]	MON 88302[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88302 minus Control) Mean (S.E.) (Range)	95% Confidence Interval[3]	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dw)						
Glutamic Acid	3.89 (0.15) (3.84 - 3.87)	4.25 (0.13) (3.98 - 4.45)	-0.36 (0.17) (-0.59 - -0.11)	-0.80, 0.083	0.091	2.73, 5.89 (3.26 - 5.43)
Glycine	1.12 (0.034) (1.11 - 1.12)	1.22 (0.030) (1.13 - 1.30)	-0.099 (0.044) (-0.18 - -0.0061)	-0.21, 0.014	0.073	0.96, 1.47 (1.01 - 1.50)
Histidine	0.62 (0.018) (0.61 - 0.62)	0.65 (0.016) (0.61 - 0.67)	-0.031 (0.022) (-0.058 - -0.00063)	-0.087, 0.025	0.214	0.47, 0.86 (0.54 - 0.80)
Isoleucine	0.90 (0.027) (0.88 - 0.90)	0.97 (0.024) (0.89 - 1.03)	-0.076 (0.031) (-0.13 - -0.0068)	-0.16, 0.0044	0.059	0.70, 1.22 (0.78 - 1.15)
Leucine	1.56 (0.052) (1.55 - 1.55)	1.70 (0.045) (1.58 - 1.80)	-0.15 (0.064) (-0.25 - -0.034)	-0.31, 0.015	0.066	1.21, 2.18 (1.36 - 2.07)
Lysine	1.37 (0.032) (1.35 - 1.36)	1.40 (0.029) (1.35 - 1.44)	-0.033 (0.036) (-0.055 - -0.0095)	-0.13, 0.059	0.397	1.02, 1.90 (1.20 - 1.68)

Table E-7. Statistical Summary of Site MNCA Canola Seed Nutrient Content for MON 88302 vs. the Conventional Control (continued)

Analytical Component (Units)[1]	MON 88302[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88302 minus Control) Mean (S.E.) (Range)	95% Confidence Interval[3]	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dw)						
Methionine	0.44 (0.020) (0.43 - 0.45)	0.44 (0.017) (0.42 - 0.48)	0.0058 (0.024) (0.011 - 0.019)	-0.056, 0.067	0.819	0.30, 0.65 (0.36 - 0.57)
Phenylalanine	0.93 (0.030) (0.92 - 0.93)	1.02 (0.026) (0.94 - 1.08)	-0.089 (0.036) (-0.17 - -0.011)	-0.18, 0.0043	0.057	0.77, 1.26 (0.84 - 1.25)
Proline	1.36 (0.045) (1.29 - 1.39)	1.40 (0.040) (1.30 - 1.46)	-0.040 (0.053) (-0.16 - 0.076)	-0.18, 0.096	0.479	0.90, 2.01 (1.12 - 1.78)
Serine	0.98 (0.038) (0.98 - 0.99)	1.08 (0.033) (1.02 - 1.16)	-0.095 (0.050) (-0.17 - -0.037)	-0.22, 0.033	0.114	0.81, 1.32 (0.88 - 1.30)
Threonine	0.95 (0.027) (0.94 - 0.94)	1.02 (0.024) (0.97 - 1.06)	-0.073 (0.031) (-0.12 - -0.027)	-0.15, 0.0074	0.067	0.82, 1.20 (0.84 - 1.22)
Tryptophan	0.22 (0.020) (0.18 - 0.24)	0.24 (0.017) (0.22 - 0.27)	-0.022 (0.026) (-0.063 - 0.024)	-0.089, 0.044	0.426	0.13, 0.35 (0.17 - 0.32)

Table E-7. statistical Summary of Site MNCA Canola Seed Nutrient Content for MON 88302 vs. the Conventional Control (continued)

Analytical Component (Units)[1]	MON 88302[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88302 minus Control) Mean (S.E.) (Range)	95% Confidence Interval[3]	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dw)						
Tyrosine	0.65 (0.019) (0.64 - 0.65)	0.71 (0.017) (0.66 - 0.75)	-0.061 (0.024) (-0.11 - -0.0055)	-0.12, 0.0017	0.054	0.57, 0.81 (0.60 - 0.84)
Valine	1.15 (0.031) (1.13 - 1.15)	1.24 (0.027) (1.15 - 1.31)	-0.091 (0.035) (-0.16 - -0.017)	-0.18, -0.00082	0.048	0.92, 1.55 (1.01 - 1.46)
Fatty Acid (% Total FA)						
16:0 Palmitic	4.27 (0.051) (4.27 - 4.28)	4.14 (0.045) (4.07 - 4.19)	0.13 (0.061) (0.087 - 0.21)	-0.029, 0.28	0.090	2.84, 5.26 (3.55 - 4.69)
16:1 Palmitoleic	0.21 (0.0030) (0.21 - 0.21)	0.24 (0.0026) (0.23 - 0.25)	-0.026 (0.0039) (-0.039 - -0.020)	-0.036, -0.016	0.001	0.17, 0.30 (0.19 - 0.27)
18:0 Stearic	1.67 (0.044) (1.65 - 1.71)	1.86 (0.038) (1.78 - 1.92)	-0.19 (0.057) (-0.26 - -0.074)	-0.33, -0.039	0.022	0.90, 3.05 (1.50 - 2.64)
18:1 Oleic	61.67 (0.59) (61.70 - 61.87)	64.86 (0.52) (63.72 - 65.52)	-3.19 (0.69) (-3.81 - -3.11)	-4.98, -1.41	0.005	56.13, 70.69 (57.86 - 68.53)

Table E-7. Statistical Summary of Site MNCA Canola Seed Nutrient Content for MON 88302 vs. the Conventional Control (continued)

Analytical Component (Units)[1]	MON 88302[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88302 minus Control) Mean (S.E.) (Range)	95% Confidence Interval[3]	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fatty Acid (% Total FA)						
18:2 Linoleic	20.20 (0.24) (20.00 - 20.32)	18.35 (0.21) (17.90 - 19.29)	1.85 (0.30) (1.86 - 2.42)	1.07, 2.63	0.001	12.60, 24.49 (14.12 - 22.57)
18:3 Linolenic	9.79 (0.58) (9.76 - 9.79)	8.40 (0.50) (8.16 - 8.64)	1.39 (0.75) (1.31 - 1.60)	-0.54, 3.32	0.122	6.96, 11.73 (7.99 - 10.94)
20:0 Arachidic	0.53 (0.013) (0.52 - 0.54)	0.57 (0.012) (0.55 - 0.60)	-0.042 (0.018) (-0.081 - -0.019)	-0.088, 0.0027	0.060	0.45, 0.80 (0.53 - 0.71)
20:1 Eicosenoic	1.08 (0.012) (1.06 - 1.09)	1.07 (0.011) (1.05 - 1.09)	0.016 (0.016) (-0.024 - 0.041)	-0.025, 0.058	0.354	0.83, 1.68 (1.04 - 1.56)
22:0 Behenic	0.27 (0.0056) (0.27 - 0.28)	0.27 (0.0049) (0.26 - 0.29)	0.0022 (0.0074) (-0.021 - 0.016)	-0.017, 0.021	0.776	0.19, 0.43 (0.27 - 0.38)
24:0 Lignoceric	0.11 (0.033) (0.049 - 0.16)	0.14 (0.029) (0.049 - 0.19)	-0.032 (0.044) (-0.14 - 0.069)	-0.15, 0.081	0.499	0.033, 0.25 (0.044 - 0.21)

Table E-7. Statistical Summary of Site MNCA Canola Seed Nutrient Content for MON 88302 vs. the Conventional Control (continued)

Analytical Component (Units)[1]	MON 88302[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88302 minus Control) Mean (S.E.) (Range)	95% Confidence Interval[3]	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fatty Acid (% Total FA)						
24:1 Nervonic	0.10 (0.025) (0.049 - 0.15)	0.099 (0.021) (0.049 - 0.12)	0.0042 (0.032) (-0.072 - 0.062)	-0.079, 0.088	0.902	0.041, 0.18 (0.044 - 0.20)
Mineral						
Calcium (g/100g dw)	0.47 (0.016) (0.46 - 0.47)	0.45 (0.014) (0.42 - 0.49)	0.018 (0.022) (-0.0037 - 0.053)	-0.038, 0.074	0.438	0.16, 0.61 (0.25 - 0.53)
Copper (mg/kg dw)	4.40 (0.074) (4.16 - 4.57)	4.11 (0.064) (4.06 - 4.18)	0.28 (0.093) (0.056 - 0.39)	0.046, 0.52	0.027	2.00, 4.43 (2.52 - 4.93)
Iron (mg/kg dw)	42.57 (1.72) (40.56 - 44.18)	50.64 (1.53) (46.23 - 54.03)	-8.07 (1.87) (-12.92 - -4.82)	-12.89, -3.25	0.007	23.39, 86.23 (39.16 - 77.92)
Magnesium (g/100g dw)	0.38 (0.0074) (0.36 - 0.40)	0.37 (0.0067) (0.36 - 0.38)	0.014 (0.0076) (0.0074 - 0.018)	-0.0049, 0.034	0.113	0.32, 0.43 (0.30 - 0.45)
Manganese (mg/kg dw)	38.70 (2.68) (37.83 - 39.93)	40.94 (2.32) (33.70 - 46.19)	-2.24 (3.54) (-8.36 - -2.11)	-11.34, 6.86	0.554	14.85, 61.05 (25.00 - 54.11)

Table E-7. Statistical Summary of Site MNCA Canola Seed Nutrient Content for MON 88302 vs. the Conventional Control (continued)

Analytical Component (Units)[1]	MON 88302[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88302 minus Control) Mean (S.E.) (Range)	95% Confidence Interval[3]	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Mineral						
Phosphorus (g/100g dw)	0.81 (0.039) (0.74 - 0.87)	0.79 (0.034) (0.72 - 0.93)	0.022 (0.044) (-0.011 - 0.098)	-0.090, 0.13	0.633	0.38, 1.06 (0.44 - 0.87)
Potassium (g/100g dw)	0.65 (0.027) (0.58 - 0.70)	0.64 (0.023) (0.60 - 0.72)	0.012 (0.035) (-0.020 - 0.098)	-0.079, 0.10	0.746	0.39, 0.96 (0.50 - 0.92)
Zinc (mg/kg dw)	39.18 (2.73) (35.19 - 45.56)	35.29 (2.41) (32.63 - 36.66)	3.90 (3.15) (-1.44 - 10.33)	-4.21, 12.00	0.271	20.19, 48.23 (22.18 - 47.61)
Vitamin (mg/100g dw)						
Vitamin E (α-tocopherol)	13.39 (0.48) (12.58 - 14.62)	10.82 (0.42) (10.15 - 11.77)	2.57 (0.57) (2.18 - 3.99)	1.11, 4.03	0.006	3.88, 17.28 (2.62 - 14.84)

[1]dw = dry weight; fw = fresh weight; FA = fatty acid.
[2] MON 88302 treated with glyphosate.
[3]Mean (S.E.) = least-square mean (standard error); CI = confidence interval.
[4]Control refers to the genetically similar, conventional control.
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial conventional references. Negative limits set to zero.

Table E-8. Statistical Summary of Site MNCA Canola Seed Anti-nutrient Content for MON 88302 vs. the Conventional Control

Analytical Component (Units)[1]	MON 88302[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88302 minus Control) Mean (S.E.) (Range)	95% Confidence Interval[3]	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Anti-nutrient						
Alkyl Glucosinolate (µmole/g dw)	4.64 (0.62) (4.35 - 4.88)	4.88 (0.54) (2.92 - 6.16)	-0.24 (0.79) (-1.48 - 1.43)	-2.28, 1.80	0.775	0, 29.02 (2.32 - 28.33)
Indolyl Glucosinolate (µmole/g dw)	4.17 (0.52) (3.79 - 4.47)	4.35 (0.45) (3.28 - 5.66)	-0.19 (0.69) (-1.42 - 0.13)	-1.96, 1.59	0.798	1.37, 6.62 (1.84 - 7.18)
Phytic Acid (% dw)	2.28 (0.12) (2.14 - 2.47)	2.36 (0.10) (2.15 - 2.77)	-0.078 (0.15) (-0.011 - 0.12)	-0.47, 0.31	0.630	0.70, 3.52 (1.10 - 2.71)
Sinapic Acid (% dw)	1.06 (0.015) (1.02 - 1.08)	0.96 (0.014) (0.94 - 0.97)	0.10 (0.015) (0.082 - 0.12)	0.063, 0.14	0.001	0.57, 1.13 (0.48 - 0.99)
Total Glucosinolate (µmole/g dw)	9.08 (0.69) (8.35 - 9.36)	9.42 (0.61) (7.15 - 10.65)	-0.33 (0.81) (-1.29 - 1.19)	-2.41, 1.74	0.696	0, 32.20 (5.52 - 31.98)

[1]dw = dry weight.
[2] MON 88302 treated with glyphosate.
[3]Mean (S.E.) = least-square mean (standard error); CI = confidence interval.
[4]Control refers to the genetically similar, conventional control.
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial conventional references. Negative limits set to zero.

Table E-9. Statistical Summary of Site NDVA Canola Seed Nutrient Content for MON 88302 vs. the Conventional Control

Analytical Component (Units)[1]	MON 88302[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88302 minus Control) Mean (S.E.) (Range)	95% Confidence Interval[3]	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Proximate (% dw)						
Ash	3.31 (0.31) (3.31 - 3.31)	3.25 (0.26) (3.20 - 3.35)	0.063 (0.40) (0.11 - 0.11)	-1.22, 1.35	0.886	3.32, 4.66 (2.98 - 4.52)
Carbohydrates	24.01 (0.95) (21.83 - 26.20)	25.49 (0.78) (24.69 - 26.01)	-1.48 (1.22) (-4.18 - 0.45)	-5.37, 2.42	0.314	23.12, 30.77 (22.53 - 29.96)
Moisture (% fw)	5.72 (0.23) (5.72 - 6.08)	5.61 (0.20) (5.24 - 6.18)	0.12 (0.22) (-0.10 - 0.090)	-0.58, 0.82	0.633	4.33, 6.91 (4.09 - 8.48)
Protein	24.66 (0.74) (23.33 - 25.98)	26.12 (0.61) (25.33 - 27.02)	-1.46 (0.96) (-1.99 - -0.028)	-4.52, 1.60	0.226	17.20, 30.08 (18.68 - 28.32)
Total Fat	48.04 (0.44) (47.20 - 48.87)	45.17 (0.36) (44.77 - 45.78)	2.87 (0.57) (1.42 - 4.10)	1.07, 4.67	0.014	39.65, 51.24 (40.71 - 50.26)
Fiber (% dw)						
Acid Detergent Fiber	15.20 (0.37) (14.53 - 15.86)	15.22 (0.31) (14.88 - 15.35)	-0.016 (0.39) (-0.62 - 0.52)	-1.26, 1.23	0.969	6.95, 23.92 (9.75 - 21.22)

Table E-9. Statistical Summary of Site NDVA Canola Seed Nutrient Content for MON 88302 vs. the Conventional Control (continued)

Analytical Component (Units)[1]	MON 88302[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88302 minus Control) Mean (S.E.) (Range)	95% Confidence Interval[3]	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fiber (% dw)						
Neutral Detergent Fiber	17.32 (0.81) (15.91 - 18.74)	17.74 (0.66) (17.16 - 18.65)	-0.42 (1.05) (-2.74 - 1.58)	-3.76, 2.93	0.718	10.07, 25.94 (10.93 - 22.75)
Total Dietary Fiber	18.71 (1.39) (17.08 - 21.08)	17.17 (1.15) (14.88 - 19.61)	1.54 (1.65) (-0.30 - 1.47)	-3.70, 6.79	0.418	13.97, 24.85 (12.64 - 26.47)
Amino Acid (% dw)						
Alanine	1.08 (0.042) (1.01 - 1.15)	1.14 (0.034) (1.11 - 1.19)	-0.061 (0.054) (-0.10 - 0.031)	-0.23, 0.11	0.344	0.77, 1.34 (0.87 - 1.27)
Arginine	1.60 (0.076) (1.47 - 1.72)	1.72 (0.062) (1.68 - 1.77)	-0.12 (0.098) (-0.21 - 0.030)	-0.43, 0.19	0.316	1.10, 1.93 (1.23 - 1.96)
Aspartic Acid	1.79 (0.067) (1.65 - 1.93)	1.83 (0.054) (1.79 - 1.89)	-0.040 (0.086) (-0.14 - 0.12)	-0.31, 0.23	0.675	1.33, 2.12 (1.42 - 2.23)
Cystine	0.70 (0.040) (0.64 - 0.73)	0.71 (0.033) (0.66 - 0.79)	-0.018 (0.046) (-0.028 - 0.037)	-0.16, 0.13	0.715	0.38, 0.83 (0.45 - 0.79)

Table E-9. Statistical Summary of Site NDVA Canola Seed Nutrient Content for MON 88302 vs. the Conventional Control (continued)

Analytical Component (Units)[1]	MON 88302[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88302 minus Control) Mean (S.E.) (Range)	95% Confidence Interval[3]	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dw)						
Glutamic Acid	4.66 (0.25) (4.25 - 5.06)	5.04 (0.20) (4.92 - 5.26)	-0.38 (0.32) (-0.68 - 0.13)	-1.41, 0.64	0.319	2.73, 5.89 (3.26 - 5.43)
Glycine	1.24 (0.048) (1.16 - 1.32)	1.33 (0.039) (1.30 - 1.38)	-0.094 (0.062) (-0.15 - 0.0093)	-0.29, 0.10	0.229	0.96, 1.47 (1.01 - 1.50)
Histidine	0.72 (0.034) (0.67 - 0.77)	0.75 (0.027) (0.73 - 0.78)	-0.032 (0.043) (-0.065 - 0.032)	-0.17, 0.11	0.509	0.47, 0.86 (0.54 - 0.80)
Isoleucine	1.01 (0.041) (0.94 - 1.08)	1.07 (0.034) (1.04 - 1.12)	-0.067 (0.053) (-0.099 - 0.0095)	-0.24, 0.10	0.299	0.70, 1.22 (0.78 - 1.15)
Leucine	1.77 (0.073) (1.64 - 1.90)	1.88 (0.059) (1.83 - 1.95)	-0.11 (0.094) (-0.19 - 0.051)	-0.40, 0.19	0.336	1.21, 2.18 (1.36 - 2.07)
Lysine	1.55 (0.066) (1.46 - 1.63)	1.60 (0.054) (1.58 - 1.65)	-0.054 (0.085) (-0.12 - 0.052)	-0.32, 0.22	0.566	1.02, 1.90 (1.20 - 1.68)

Table E-9. Statistical Summary of Site NDVA Canola Seed Nutrient Content for MON 88302 vs. the Conventional Control (continued)

Analytical Component (Units)[1]	MON 88302[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88302 minus Control) Mean (S.E.) (Range)	95% Confidence Interval[3]	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dw)						
Methionine	0.52 (0.019) (0.49 - 0.54)	0.53 (0.016) (0.50 - 0.56)	-0.0059 (0.020) (-0.013 - 0.0079)	-0.068, 0.056	0.782	0.30, 0.65 (0.36 - 0.57)
Phenylalanine	1.04 (0.038) (0.97 - 1.11)	1.11 (0.031) (1.08 - 1.15)	-0.066 (0.049) (-0.12 - 0.031)	-0.22, 0.091	0.272	0.77, 1.26 (0.84 - 1.25)
Proline	1.59 (0.065) (1.46 - 1.71)	1.68 (0.053) (1.62 - 1.73)	-0.086 (0.083) (-0.16 - 0.041)	-0.35, 0.18	0.377	0.90, 2.01 (1.12 - 1.78)
Serine	1.09 (0.036) (1.05 - 1.14)	1.16 (0.029) (1.13 - 1.18)	-0.064 (0.046) (-0.12 - 0.0094)	-0.21, 0.083	0.258	0.81, 1.32 (0.88 - 1.30)
Threonine	1.05 (0.035) (0.99 - 1.11)	1.09 (0.029) (1.06 - 1.12)	-0.037 (0.045) (-0.065 - 0.020)	-0.18, 0.11	0.470	0.82, 1.20 (0.84 - 1.22)
Tryptophan	0.26 (0.023) (0.25 - 0.26)	0.26 (0.019) (0.21 - 0.31)	0.0021 (0.025) (0.0061 - 0.036)	-0.079, 0.083	0.938	0.13, 0.35 (0.17 - 0.32)

Table E-9. Statistical Summary of Site NDVA Canola Seed Nutrient Content for MON 88302 vs. the Conventional Control (continued)

Analytical Component (Units)[1]	MON 88302[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88302 minus Control) Mean (S.E.) (Range)	95% Confidence Interval[3]	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dw)						
Tyrosine	0.71 (0.023) (0.67 - 0.75)	0.74 (0.019) (0.72 - 0.77)	-0.031 (0.029) (-0.060 - 0.025)	-0.13, 0.062	0.364	0.57, 0.81 (0.60 - 0.84)
Valine	1.29 (0.050) (1.21 - 1.37)	1.36 (0.041) (1.32 - 1.41)	-0.073 (0.064) (-0.12 - 0.020)	-0.28, 0.13	0.340	0.92, 1.55 (1.01 - 1.46)
Fatty Acid (% Total FA)						
16:0 Palmitic	3.98 (0.065) (3.95 - 4.02)	3.95 (0.053) (3.94 - 3.96)	0.036 (0.084) (-0.016 - 0.083)	-0.23, 0.30	0.699	2.84, 5.26 (3.55 - 4.69)
16:1 Palmitoleic	0.20 (0.0057) (0.20 - 0.20)	0.22 (0.0046) (0.22 - 0.23)	-0.025 (0.0068) (-0.029 - -0.023)	-0.047, -0.0031	0.036	0.17, 0.30 (0.19 - 0.27)
18:0 Stearic	1.77 (0.033) (1.71 - 1.84)	2.11 (0.027) (2.10 - 2.12)	-0.34 (0.042) (-0.41 - -0.28)	-0.47, -0.20	0.004	0.90, 3.05 (1.50 - 2.64)
18:1 Oleic	65.14 (0.35) (64.90 - 65.20)	68.38 (0.29) (68.11 - 68.44)	-3.24 (0.37) (-3.24 - -3.21)	-4.43, -2.05	0.003	56.13, 70.69 (57.86 - 68.53)

Table E-9. Statistical Summary of Site NDVA Canola Seed Nutrient Content for MON 88302 vs. the Conventional Control (continued)

Analytical Component (Units)[1]	MON 88302[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88302 minus Control) Mean (S.E.) (Range)	95% Confidence Interval[3]	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fatty Acid (% Total FA)						
18:2 Linoleic	17.86 (0.31) (17.78 - 18.02)	15.71 (0.25) (15.72 - 15.77)	2.15 (0.36) (2.06 - 2.26)	1.00, 3.29	0.009	12.60, 24.49 (14.12 - 22.57)
18:3 Linolenic	8.82 (0.070) (8.71 - 8.94)	7.31 (0.057) (7.19 - 7.40)	1.51 (0.091) (1.35 - 1.54)	1.22, 1.80	<0.001	6.96, 11.73 (7.99 - 10.94)
20:0 Arachidic	0.57 (0.0047) (0.56 - 0.57)	0.65 (0.0046) (0.64 - 0.65)	-0.082 (0.0023) (-0.085 - -0.077)	-0.089, -0.074	<0.001	0.45, 0.80 (0.53 - 0.71)
20:1 Eicosenoic	1.15 (0.011) (1.13 - 1.17)	1.16 (0.0088) (1.15 - 1.18)	-0.0098 (0.014) (-0.042 - 0.020)	-0.054, 0.035	0.531	0.83, 1.68 (1.04 - 1.56)
22:0 Behenic	0.27 (0.0036) (0.27 - 0.27)	0.30 (0.0029) (0.30 - 0.30)	-0.029 (0.0044) (-0.031 - -0.027)	-0.043, -0.015	0.007	0.19, 0.43 (0.27 - 0.38)
24:0 Lignoceric	0.17 (0.053) (0.16 - 0.18)	0.16 (0.043) (0.049 - 0.22)	0.012 (0.068) (-0.045 - 0.11)	-0.20, 0.23	0.873	0.033, 0.25 (0.044 - 0.21)

Table E-9. Statistical Summary of Site NDVA Canola Seed Nutrient Content for MON 88302 vs. the Conventional Control (continued)

Analytical Component (Units)[1]	MON 88302[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88302 minus Control) Mean (S.E.) (Range)	95% Confidence Interval[3]	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fatty Acid (% Total FA)						
24:1 Nervonic	0.12 (0.028) (0.11 - 0.13)	0.090 (0.023) (0.049 - 0.12)	0.031 (0.037) (-0.0061 - 0.081)	-0.086, 0.15	0.460	0.041, 0.18 (0.044 - 0.20)
Mineral						
Calcium (g/100g dw)	0.34 (0.049) (0.30 - 0.37)	0.31 (0.040) (0.28 - 0.34)	0.030 (0.063) (-0.038 - 0.068)	-0.17, 0.23	0.663	0.16, 0.61 (0.25 - 0.53)
Copper (mg/kg dw)	3.80 (0.092) (3.72 - 3.89)	3.53 (0.076) (3.33 - 3.67)	0.28 (0.11) (0.16 - 0.22)	-0.084, 0.64	0.092	2.00, 4.43 (2.52 - 4.93)
Iron (mg/kg dw)	51.55 (4.68) (46.78 - 56.32)	63.44 (3.82) (60.79 - 67.18)	-11.89 (6.04) (-20.41 - -6.03)	-31.12, 7.34	0.143	23.39, 86.23 (39.16 - 77.92)
Magnesium (g/100g dw)	0.36 (0.012) (0.34 - 0.37)	0.35 (0.0099) (0.33 - 0.37)	0.0074 (0.016) (-0.012 - 0.043)	-0.043, 0.058	0.668	0.32, 0.43 (0.30 - 0.45)
Manganese (mg/kg dw)	50.88 (6.56) (46.85 - 51.55)	50.07 (5.45) (47.11 - 50.97)	0.81 (7.57) (-0.26 - 0.58)	-23.29, 24.90	0.921	14.85, 61.05 (25.00 - 54.11)

Table E-9. Statistical Summary of Site NDVA Canola Seed Nutrient Content for MON 88302 vs. the Conventional Control (continued)

Analytical Component (Units)[1]	MON 88302[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88302 minus Control) Mean (S.E.) (Range)	95% Confidence Interval[3]	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Mineral						
Phosphorus (g/100g dw)	0.57 (0.077) (0.56 - 0.59)	0.57 (0.063) (0.56 - 0.58)	0.0026 (0.094) (0.0027 - 0.014)	-0.30, 0.30	0.979	0.38, 1.06 (0.44 - 0.87)
Potassium (g/100g dw)	0.48 (0.037) (0.48 - 0.49)	0.54 (0.030) (0.53 - 0.54)	-0.053 (0.048) (-0.053 - -0.048)	-0.20, 0.099	0.346	0.39, 0.96 (0.50 - 0.92)
Zinc (mg/kg dw)	30.46 (1.73) (29.81 - 31.08)	32.41 (1.43) (28.89 - 34.23)	-1.94 (2.07) (-3.15 - 0.93)	-8.52, 4.63	0.416	20.19, 48.23 (22.18 - 47.61)
Vitamin (mg/100g dw)						
Vitamin E (α-tocopherol)	15.89 (0.88) (15.23 - 16.55)	9.43 (0.72) (8.46 - 10.20)	6.45 (1.14) (5.03 - 8.09)	2.82, 10.08	0.010	3.88, 17.28 (2.62 - 14.84)

[1]dw = dry weight; fw = fresh weight; FA = fatty acid.
[2] MON 88302 treated with glyphosate.
[3]Mean (S.E.) = least-square mean (standard error); CI = confidence interval.
[4]Control refers to the genetically similar, conventional control.
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial conventional references. Negative limits set to zero.

Table E-10. Statistical Summary of Site NDVA Canola Seed Anti-nutrient Content for MON 88302 vs. the Conventional Control

Analytical Component (Units)[1]	MON 88302[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88302 minus Control) Mean (S.E.) (Range)	95% Confidence Interval[3]	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Anti-nutrient						
Alkyl Glucosinolate (µmole/g dw)	3.90 (0.65) (3.74 - 3.88)	4.22 (0.54) (2.45 - 5.22)	-0.32 (0.76) (-1.34 - 1.29)	-2.73, 2.10	0.705	0, 29.02 (2.32 - 28.33)
Indolyl Glucosinolate (µmole/g dw)	4.51 (0.57) (3.99 - 4.48)	3.40 (0.49) (1.83 - 4.23)	1.11 (0.58) (0.25 - 2.16)	-0.74, 2.96	0.152	1.37, 6.62 (1.84 - 7.18)
Phytic Acid (% dw)	1.58 (0.097) (1.41 - 1.57)	1.59 (0.088) (1.46 - 1.68)	-0.0050 (0.082) (-0.052 - -0.012)	-0.27, 0.26	0.954	0.70, 3.52 (1.10 - 2.71)
Sinapic Acid (% dw)	1.02 (0.027) (1.00 - 1.04)	0.83 (0.025) (0.83 - 0.88)	0.19 (0.018) (0.18 - 0.21)	0.14, 0.25	0.001	0.57, 1.13 (0.48 - 0.99)
Total Glucosinolate (µmole/g dw)	8.59 (1.20) (7.96 - 8.57)	7.72 (1.00) (4.38 - 9.61)	0.87 (1.33) (-1.04 - 3.58)	-3.37, 5.10	0.560	0, 32.20 (5.52 - 31.98)

[1]dw = dry weight.
[2] MON 88302 treated with glyphosate.
[3]Mean (S.E.) = least-square mean (standard error); CI = confidence interval.
[4]Control refers to the genetically similar, conventional control.
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial conventional references. Negative limits set to zero.

Table E-11. Statistical Summary of Site SKSA Canola Seed Nutrient Content for MON 88302 vs. the Conventional Control

Analytical Component (Units)[1]	MON 88302[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88302 minus Control) Mean (S.E.) (Range)	95% Confidence Interval[3]	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Proximate (% dw)						
Ash	3.95 (0.18) (3.68 - 4.44)	4.01 (0.17) (3.70 - 4.33)	-0.058 (0.10) (-0.20 - 0.12)	-0.34, 0.22	0.594	3.32, 4.66 (2.98 - 4.52)
Carbohydrates	23.46 (0.51) (23.11 - 23.84)	25.05 (0.44) (23.91 - 26.81)	-1.59 (0.59) (-2.97 - -0.39)	-3.23, 0.056	0.055	23.12, 30.77 (22.53 - 29.96)
Moisture (% fw)	4.36 (0.21) (3.90 - 4.82)	4.69 (0.18) (4.41 - 4.88)	-0.33 (0.28) (-0.75 - 0.010)	-1.12, 0.45	0.306	4.33, 6.91 (4.09 - 8.48)
Protein	23.82 (0.55) (23.62 - 24.58)	22.14 (0.50) (21.03 - 24.16)	1.68 (0.55) (0.42 - 2.50)	0.15, 3.21	0.038	17.20, 30.08 (18.68 - 28.32)
Total Fat	48.83 (0.65) (47.91 - 49.22)	48.81 (0.59) (46.96 - 50.24)	0.022 (0.64) (-1.02 - 0.95)	-1.76, 1.80	0.974	39.65, 51.24 (40.71 - 50.26)
Fiber (% dw)						
Acid Detergent Fiber	10.40 (0.63) (9.19 - 11.50)	9.85 (0.61) (8.94 - 10.78)	0.54 (0.35) (-0.011 - 1.18)	-0.42, 1.51	0.193	6.95, 23.92 (9.75 - 21.22)

Table E-11. Statistical Summary of Site SKSA Canola Seed Nutrient Content for MON 88302 vs. the Conventional Control (continued)

Analytical Component (Units)[1]	MON 88302[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88302 minus Control) Mean (S.E.) (Range)	95% Confidence Interval[3]	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fiber (% dw)						
Neutral Detergent Fiber	11.61 (0.73) (9.48 - 12.75)	12.59 (0.67) (11.56 - 13.91)	-0.98 (0.63) (-2.08 - 0.013)	-2.72, 0.77	0.194	10.07, 25.94 (10.93 - 22.75)
Total Dietary Fiber	18.68 (0.54) (17.17 - 19.24)	17.21 (0.48) (16.57 - 17.89)	1.48 (0.56) (0.60 - 2.52)	-0.075, 3.03	0.057	13.97, 24.85 (12.64 - 26.47)
Amino Acid (% dw)						
Alanine	1.05 (0.033) (1.02 - 1.07)	1.01 (0.028) (0.93 - 1.10)	0.040 (0.043) (-0.031 - 0.069)	-0.080, 0.16	0.405	0.77, 1.34 (0.87 - 1.27)
Arginine	1.33 (0.052) (1.27 - 1.38)	1.39 (0.045) (1.29 - 1.55)	-0.062 (0.069) (-0.18 - -0.018)	-0.26, 0.13	0.420	1.10, 1.93 (1.23 - 1.96)
Aspartic Acid	1.45 (0.037) (1.40 - 1.50)	1.53 (0.032) (1.46 - 1.65)	-0.085 (0.049) (-0.15 - -0.084)	-0.22, 0.052	0.159	1.33, 2.12 (1.42 - 2.23)
Cystine	0.55 (0.022) (0.53 - 0.57)	0.55 (0.019) (0.49 - 0.62)	-0.0025 (0.025) (-0.050 - 0.053)	-0.072, 0.067	0.923	0.38, 0.83 (0.45 - 0.79)

Table E-11. Statistical Summary of Site SKSA Canola Seed Nutrient Content for MON 88302 vs. the Conventional Control (continued)

Analytical Component (Units)[1]	MON 88302[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88302 minus Control) Mean (S.E.) (Range)	95% Confidence Interval[3]	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dw)						
Glutamic Acid	3.84 (0.14) (3.66 - 3.97)	3.98 (0.12) (3.65 - 4.39)	-0.14 (0.18) (-0.50 - 0.094)	-0.64, 0.36	0.493	2.73, 5.89 (3.26 - 5.43)
Glycine	1.05 (0.023) (1.02 - 1.07)	1.08 (0.020) (1.01 - 1.15)	-0.026 (0.030) (-0.084 - -0.016)	-0.11, 0.058	0.436	0.96, 1.47 (1.01 - 1.50)
Histidine	0.59 (0.015) (0.57 - 0.60)	0.61 (0.013) (0.57 - 0.65)	-0.016 (0.019) (-0.058 - 0.0030)	-0.070, 0.037	0.443	0.47, 0.86 (0.54 - 0.80)
Isoleucine	0.91 (0.021) (0.89 - 0.93)	0.90 (0.018) (0.84 - 0.96)	0.011 (0.028) (-0.049 - 0.041)	-0.067, 0.088	0.716	0.70, 1.22 (0.78 - 1.15)
Leucine	1.61 (0.045) (1.55 - 1.65)	1.58 (0.039) (1.46 - 1.71)	0.029 (0.059) (-0.073 - 0.085)	-0.13, 0.19	0.644	1.21, 2.18 (1.36 - 2.07)
Lysine	1.33 (0.037) (1.27 - 1.37)	1.37 (0.032) (1.30 - 1.48)	-0.034 (0.050) (-0.12 - 0.024)	-0.17, 0.10	0.527	1.02, 1.90 (1.20 - 1.68)

Table E-11. Statistical Summary of Site SKSA Canola Seed Nutrient Content for MON 88302 vs. the Conventional Control (continued)

Analytical Component (Units)[1]	MON 88302[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88302 minus Control) Mean (S.E.) (Range)	95% Confidence Interval[3]	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dw)						
Methionine	0.45 (0.015) (0.44 - 0.47)	0.44 (0.013) (0.40 - 0.49)	0.0082 (0.016) (-0.022 - 0.034)	-0.037, 0.054	0.643	0.30, 0.65 (0.36 - 0.57)
Phenylalanine	0.96 (0.023) (0.93 - 0.98)	0.94 (0.020) (0.87 - 1.01)	0.026 (0.031) (-0.028 - 0.044)	-0.059, 0.11	0.443	0.77, 1.26 (0.84 - 1.25)
Proline	1.39 (0.035) (1.34 - 1.42)	1.44 (0.030) (1.35 - 1.52)	-0.050 (0.046) (-0.13 - 0.0026)	-0.18, 0.077	0.335	0.90, 2.01 (1.12 - 1.78)
Serine	0.97 (0.025) (0.93 - 0.99)	0.99 (0.022) (0.94 - 1.06)	-0.022 (0.033) (-0.067 - -0.0013)	-0.11, 0.070	0.546	0.81, 1.32 (0.88 - 1.30)
Threonine	0.89 (0.022) (0.86 - 0.92)	0.93 (0.019) (0.88 - 0.99)	-0.038 (0.029) (-0.072 - -0.031)	-0.12, 0.043	0.263	0.82, 1.20 (0.84 - 1.22)
Tryptophan	0.20 (0.0091) (0.19 - 0.22)	0.22 (0.0082) (0.21 - 0.24)	-0.018 (0.0093) (-0.035 - 0.0041)	-0.043, 0.0084	0.134	0.13, 0.35 (0.17 - 0.32)

Table E-11. Statistical Summary of Site SKSA Canola Seed Nutrient Content for MON 88302 vs. the Conventional Control (continued)

Analytical Component (Units)[1]	MON 88302[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88302 minus Control) Mean (S.E.) (Range)	95% Confidence Interval[3]	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dw)						
Tyrosine	0.64 (0.014) (0.61 - 0.65)	0.64 (0.012) (0.61 - 0.68)	-0.0084 (0.018) (-0.037 - 0.0079)	-0.060, 0.043	0.672	0.57, 0.81 (0.60 - 0.84)
Valine	1.16 (0.025) (1.13 - 1.19)	1.15 (0.022) (1.08 - 1.23)	0.0090 (0.034) (-0.073 - 0.046)	-0.085, 0.10	0.801	0.92, 1.55 (1.01 - 1.46)
Fatty Acid (% Total FA)						
16:0 Palmitic	4.51 (0.047) (4.46 - 4.57)	4.07 (0.042) (4.05 - 4.10)	0.44 (0.049) (0.41 - 0.48)	0.31, 0.58	<0.001	2.84, 5.26 (3.55 - 4.69)
16:1 Palmitoleic	0.26 (0.0047) (0.25 - 0.26)	0.25 (0.0043) (0.24 - 0.25)	0.0062 (0.0044) (0.0043 - 0.0074)	-0.0061, 0.019	0.235	0.17, 0.30 (0.19 - 0.27)
18:0 Stearic	1.66 (0.055) (1.54 - 1.72)	2.08 (0.049) (1.91 - 2.19)	-0.42 (0.057) (-0.48 - -0.35)	-0.58, -0.26	0.001	0.90, 3.05 (1.50 - 2.64)
18:1 Oleic	61.91 (0.55) (60.51 - 62.29)	65.69 (0.51) (64.73 - 66.86)	-3.78 (0.47) (-4.30 - -2.59)	-5.08, -2.48	0.001	56.13, 70.69 (57.86 - 68.53)

Table E-11. Statistical Summary of Site SKSA Canola Seed Nutrient Content for MON 88302 vs. the Conventional Control (continued)

Analytical Component (Units)[1]	MON 88302[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88302 minus Control) Mean (S.E.) (Range)	95% Confidence Interval[3]	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fatty Acid (% Total FA)						
18:2 Linoleic	18.49 (0.37) (18.08 - 19.48)	17.22 (0.34) (16.64 - 18.01)	1.27 (0.34) (0.40 - 1.90)	0.33, 2.20	0.019	12.60, 24.49 (14.12 - 22.57)
18:3 Linolenic	10.78 (0.23) (10.39 - 11.23)	8.38 (0.20) (7.94 - 8.99)	2.40 (0.25) (2.08 - 2.64)	1.72, 3.09	<0.001	6.96, 11.73 (7.99 - 10.94)
20:0 Arachidic	0.54 (0.0079) (0.52 - 0.55)	0.62 (0.0069) (0.59 - 0.63)	-0.082 (0.0090) (-0.091 - -0.070)	-0.11, -0.057	<0.001	0.45, 0.80 (0.53 - 0.71)
20:1 Eicosenoic	1.24 (0.015) (1.22 - 1.26)	1.13 (0.013) (1.10 - 1.17)	0.11 (0.020) (0.050 - 0.14)	0.052, 0.16	0.005	0.83, 1.68 (1.04 - 1.56)
22:0 Behenic	0.28 (0.0031) (0.28 - 0.29)	0.29 (0.0027) (0.28 - 0.30)	-0.0088 (0.0037) (-0.014 - 0.0025)	-0.019, 0.0014	0.073	0.19, 0.43 (0.27 - 0.38)
24:0 Lignoceric	0.20 (0.029) (0.20 - 0.23)	0.15 (0.026) (0.045 - 0.22)	0.050 (0.030) (-0.016 - 0.062)	-0.032, 0.13	0.163	0.033, 0.25 (0.044 - 0.21)

Table E-11. Statistical Summary of Site SKSA Canola Seed Nutrient Content for MON 88302 vs. the Conventional Control (continued)

Analytical Component (Units)[1]	MON 88302[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88302 minus Control) Mean (S.E.) (Range)	95% Confidence Interval[3]	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fatty Acid (% Total FA)						
24:1 Nervonic	0.17 (0.020) (0.16 - 0.20)	0.12 (0.018) (0.045 - 0.16)	0.055 (0.021) (0.034 - 0.055)	-0.0021, 0.11	0.055	0.041, 0.18 (0.044 - 0.20)
Mineral						
Calcium (g/100g dw)	0.41 (0.015) (0.40 - 0.42)	0.44 (0.013) (0.40 - 0.47)	-0.031 (0.020) (-0.068 - 0.0021)	-0.086, 0.025	0.199	0.16, 0.61 (0.25 - 0.53)
Copper (mg/kg dw)	3.52 (0.16) (3.27 - 3.85)	3.23 (0.15) (2.96 - 3.48)	0.29 (0.18) (-0.21 - 0.57)	-0.21, 0.78	0.181	2.00, 4.43 (2.52 - 4.93)
Iron (mg/kg dw)	63.21 (8.79) (55.62 - 69.61)	59.66 (7.61) (50.11 - 77.74)	3.56 (11.63) (-13.33 - 14.87)	-28.72, 35.84	0.774	23.39, 86.23 (39.16 - 77.92)
Magnesium (g/100g dw)	0.36 (0.017) (0.31 - 0.39)	0.36 (0.016) (0.34 - 0.39)	-0.0044 (0.014) (-0.032 - 0.026)	-0.042, 0.033	0.764	0.32, 0.43 (0.30 - 0.45)
Manganese (mg/kg dw)	41.77 (2.44) (38.56 - 47.24)	34.73 (2.12) (33.12 - 37.61)	7.04 (3.03) (0.95 - 12.63)	-1.37, 15.45	0.080	14.85, 61.05 (25.00 - 54.11)

Table E-11. Statistical Summary of Site SKSA Canola Seed Nutrient Content for MON 88302 vs. the Conventional Control (continued)

Analytical Component (Units)[1]	MON 88302[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88302 minus Control) Mean (S.E.) (Range)	95% Confidence Interval[3]	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Mineral						
Phosphorus (g/100g dw)	0.68 (0.051) (0.57 - 0.80)	0.74 (0.048) (0.67 - 0.78)	-0.057 (0.043) (-0.095 - 0.040)	-0.18, 0.062	0.254	0.38, 1.06 (0.44 - 0.87)
Potassium (g/100g dw)	0.82 (0.030) (0.77 - 0.90)	0.71 (0.030) (0.67 - 0.80)	0.11 (0.012) (0.084 - 0.14)	0.077, 0.14	<0.001	0.39, 0.96 (0.50 - 0.92)
Zinc (mg/kg dw)	41.58 (2.06) (39.33 - 45.49)	33.10 (1.90) (29.75 - 40.66)	8.48 (1.84) (4.84 - 11.44)	3.36, 13.60	0.010	20.19, 48.23 (22.18 - 47.61)
Vitamin (mg/100g dw)						
Vitamin E (α-tocopherol)	1.49 (1.08) (1.30 - 1.66)	6.91 (0.94) (3.33 - 9.22)	-5.43 (1.43) (-6.92 - -1.67)	-9.40, -1.45	0.019	3.88, 17.28 (2.62 - 14.84)

[1]dw = dry weight; fw = fresh weight; FA = fatty acid.
[2] MON 88302 treated with glyphosate.
[3]Mean (S.E.) = least-square mean (standard error); CI = confidence interval.
[4]Control refers to the genetically similar, conventional control.
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial conventional references. Negative limits set to zero.

Table E-12. Statistical Summary of Site SKSA Canola Seed Anti-nutrient Content for MON 88302 vs. the Conventional Control

Analytical Component (Units)[1]	MON 88302[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (MON 88302 minus Control) Mean (S.E.) (Range)	95% Confidence Interval[3]	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Anti-nutrient						
Alkyl Glucosinolate (µmole/g dw)	1.61 (0.71) (1.19 - 2.17)	5.82 (0.63) (4.87 - 8.28)	-4.21 (0.78) (-6.11 - -3.28)	-6.36, -2.06	0.005	0, 29.02 (2.32 - 28.33)
Indolyl Glucosinolate (µmole/g dw)	0.86 (0.34) (0.49 - 1.31)	3.30 (0.32) (2.68 - 4.35)	-2.44 (0.29) (-3.05 - -2.19)	-3.24, -1.64	0.001	1.37, 6.62 (1.84 - 7.18)
Phytic Acid (% dw)	1.58 (0.20) (1.20 - 1.91)	1.95 (0.18) (1.69 - 2.20)	-0.37 (0.24) (-0.62 - 0.22)	-1.03, 0.29	0.191	0.70, 3.52 (1.10 - 2.71)
Sinapic Acid (% dw)	0.22 (0.059) (0.16 - 0.28)	0.81 (0.051) (0.65 - 0.95)	-0.60 (0.075) (-0.76 - -0.49)	-0.80, -0.39	0.001	0.57, 1.13 (0.48 - 0.99)
Total Glucosinolate (µmole/g dw)	2.53 (1.02) (1.73 - 3.51)	9.22 (0.93) (7.85 - 12.72)	-6.69 (1.02) (-9.21 - -5.78)	-9.51, -3.86	0.002	0, 32.20 (5.52 - 31.98)

[1]dw = dry weight.
[2] MON 88302 treated with glyphosate.
[3]Mean (S.E.) = least-square mean (standard error); CI = confidence interval.
[4]Control refers to the genetically similar, conventional control.
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial conventional references. Negative limits set to zero.

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Appendix F: Materials, Methods and Results for Seed Dormancy and Germination Assessment of MON 88302

F.1. Materials

Starting seed of MON 88302, the conventional control and commercial reference varieties, were produced in the field in Grand Forks County, ND in 2009 (Table F-1).

F.2. Characterization of the Materials

For the MON 88302, conventional control and commercial reference varieties starting seed lots, the presence or absence of MON 88302 was confirmed by event-specific polymerase chain reaction analyses.

F.3. Germination Testing Facility and Experimental Methods

Seed dormancy and germination evaluations were conducted at BioDiagnostics, Inc. in River Falls, WI. The principal investigator is qualified to conduct seed dormancy and germination testing consistent with the standards established by the Association of Official Seed Analysts (AOSA), a seed trade association (AOSA, 2009a; AOSA, 2009b).

Seed lots of MON 88302, the conventional control and four commercial reference varieties were tested under six different temperature regimes. Six germination chambers were used in this study, and each chamber was maintained with one of the six temperature regimes: constant temperature of approximately 5, 15, 25, 30 or alternating temperatures of approximately 5/25, or 15/25 °C. The constant temperature regimes were maintained with 16 h of dark and 8 h of light and the alternating temperatures regimes were maintained for 16 h at the lower temperature in the dark and 8 h at the higher temperature in the light. The temperature inside each germination chamber was monitored and recorded every 15 min. throughout the duration of the study. Starting seed for each entry was treated with Helix XTra (insecticide 20.7% thiamethoxam and fungicides 1.25% difenoconazole, 0.40% mefenoxam and 0.13% fludioxonil) at the recommended rate of 23 fl. oz. per 100 lbs of seed. Four moist blotters in a 9" x 6" germination box for each entry were prepared per facility SOPs for each temperature regime. A target of 100 seeds per entry were placed in a germination tray on moist blotters (one entry per germination tray) using a vacuum planting system. Four replicates of each entry of MON 88302, conventional control and commercial reference varieties were arranged in a randomized complete block design within each germination chamber for each temperature regime.

A description of each germination characteristic evaluated, and the timing of evaluations are presented in Table F-2. The types of data collected depended on the temperature regime. Each germination tray in the AOSA-recommended temperature regime (15 °C/25 °C) was assessed periodically during the study for normal germinated, abnormal germinated, dead (visual or ungerminated nonviable) and dormant (ungerminated viable). AOSA only provides guidelines (AOSA, 2009a) for testing seed under a recommended temperature (15 °C/25 °C). Additional temperature regimes were

included in this study to test diverse environmental conditions. Seeds in the five additional temperature regimes (5, 15, 25, 30, and 5/25 °C) were assessed 7 and 14 days after planting (DAP) and categorized as either germinated, dead, viable non-dormant or dormant. For the additional temperature regimes, no distinction was made between normal and abnormal germinated seed. Any seedling with a radicle of 1 mm or more was classified as germinated. The distinction between normal and abnormal germinated seedlings was not made because emergence and/or development of essential structures of seedlings that otherwise would be categorized as "normal germinated" under the AOSA-recommended temperature conditions may not be so at other temperatures. Fourteen DAP seeds that had not germinated were moved to the AOSA recommended temperature regime (15 °C/25 °C; 16 h dark and 8 h light) for 7 days. This additional seven day treatment was included to allow seeds that did not germinate under sub-recommended temperatures to germinate under the AOSA-recommended temperature. Twenty-one DAP (7 days at the AOSA temperature) germinated seeds were counted as "viable non-dormant" seeds. Ungerminated seeds were subject to a tetrazolium (Tz) test to assess for dormant and dead seed. Dead and dormant seed were defined the same as in the AOSA temperature regime.

F.4. Statistical Analysis

Analysis of variance (ANOVA) was conducted according to a randomized complete block design with four replicates using SAS (version 9). MON 88302 was compared to the conventional control for germination characteristics. The level of statistical significance was predetermined to be 5% ($\alpha = 0.05$). MON 88302 was not statistically compared to the commercial reference varieties and no comparisons were made across temperature regimes. The minimum and maximum mean values (reference range) were determined from the commercial reference varieties.

Table F-1. Starting Seed of MON 88302, Control and Commercial Canola Reference Varieties Used in Dormancy Assessment

Material	Material Substance Type	Phenotype	Monsanto Lot #
MON 88302	Test	Glyphosate-tolerant	11263712
Ebony	Control	Conventional	11263709
Q2	Reference	Conventional	11263207
InVigor 5550	Reference	Glufosinate-tolerant	11263706
71-45 RR	Reference	Glyphosate-tolerant	11263710
46A65	Reference	Conventional	11263707

Table F-2. Dormancy and Germination Characteristics of MON 88302 and the Conventional Control Seed

Evaluation Timing [1]	Temperature Regimes and Seed Characteristics Evaluated	
	AOSA [2, 3]	Additional Temperatures [4]
	15/25 °C	5, 15, 25, 30, 5/25 °C followed by 15/25 °C
7 DAP [5]	Normal germinated Abnormal germinated Dead	Germinated Dead
14 DAP [5]	Dormant [4] Dead	Germinated Dead
21 DAP [5]	No Data Collected	Viable non-dormant Dormant Dead

[1] Seed in the AOSA recommended (15/25 °C) temperature regime were evaluated 7 and 14 days after planting, while seed in the additional temperatures regimes were evaluated on Days 7, 14 and 21 days after planting. Ungerminated seeds in the AOSA temperature regime on day 14 were subject to tetrazolium test (Tz) to determine viability. Ungerminated seeds in the additional temperature regimes on day 14 were moved to the AOSA recommended (15/25 °C) temperature regime for 7 days (21 days after planting) and then subject to tetrazolium test (Tz) to determine viability.

[2] The Association of Official Seed Analysts.

[3] AOSA Category definitions: *Normal germinated seed*: Seedlings that exhibited normal developmental characteristics and possessed both a root and a shoot. *Abnormal germinated seed*: Seedlings that could not be classified as normal germinated (*i.e.*, insufficient root and shoot development, lack of a shoot, shoot with deep cracks or lesions or exhibited mechanical damage). *Dead seed*: Seeds that had visibly deteriorated and had become soft to the touch, or ungerminated seeds that when tested with Tz had a negative result. *Dormant seed*: Ungerminated seeds that when tested with Tz had a positive result.

[4] Non-AOSA category definitions: *Germinated*: Any seedling with a radical of 1 mm or more was classified as germinated. *Viable and not dormant:* Ungerminated seeds in the additional temperature regimes at day 14 that when moved to the AOSA temperature regime germinated. *Dead seed*: Seeds that had visibly deteriorated and had become soft to the touch or ungerminated seeds that when tested with Tz had a negative result. *Dormant seed*: Ungerminated seeds that when tested with Tz had a positive result.

[5] DAP; Days after planting.

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Appendix G: Materials, Methods and Individual Site Results from Phenotypic, Agronomic and Environmental Interaction Assessment of MON 88302 under Field Conditions

G.1. Materials

The materials for phenotypic assessment included: MON 88302, a conventional control, and 24 unique, commercial reference varieties. The list of varieties planted at each of 17 sites is presented in Table G-1.

G.2. Characterization of the Materials

For the MON 88302 and conventional control starting seed lots, the presence or absence of the glyphosate-tolerance trait was confirmed by event-specific polymerase chain reaction analyses.

G.3. Field Sites and Plot Design

Data were collected at eight field sites in the U.S. and nine sites in Canada during 2009 (Section VII, Table VII-3). These 17 locations provided a diverse range of environmental and agronomic conditions representative of commercial canola production areas in North America. The researchers at each field site were familiar with the growth, production and evaluation of canola characteristics.

The experiment was established at each of the 17 sites in a randomized complete block design with four replicates. Variation in equipment and planting methods among the sites meant some variation in seeding rate occurred. However, the seeding rates were within commercially acceptable ranges for canola (NDSU, 2005). At sites with arthropod collections (MBMI, MNPY, NDBI, and SKSA), the seeding rates were approximately 12 to 17 seeds per ft^2 over an area for each plot of 551 to 754 ft^2. At the remaining sites, the seeding rate was approximately 12 to 18 seeds per ft^2 over an area for each plot of 85 to 131 ft^2 (Table G-2).

At sites without arthropod collection, each plot was planted with a single pass of the seeder drill. One additional seeder pass was planted at the beginning and end of each replicate with a commercial canola variety. The purpose of the planted borders was to minimize edge effects. Phenotypic and qualitative environmental interactions data were collected from the middle of the seeder pass in each plot.

At sites with arthropod collection, each plot was planted with four passes of the seeder drill. Two additional seeder passes were planted at the beginning and end of each replicate with a commercial canola variety to minimize edge effects. Alleys between replicates and in the front and back of replicate 1 and 4 were planted with a commercial canola variety. The purpose of the planted border areas was to create a continuous canola stand across the study area to ensure collection of more robust arthropod abundance data. The four seeder passes in each plot were dedicated to specific types of data collection. Seeder pass 1 was used as a buffer. Seeder pass 2 was designated for the collection of the

phenotypic and qualitative environmental interactions data. Seeder pass 3 was used for the collection of the quantitative environmental interactions data. Seeder pass 4 was used to collect quantitative shattering data.

G.4. Planting and Field Operations

Field and planting information are listed in Table G-2. Agronomic practices used to prepare and maintain each study site were characteristic of those used in each respective geographic region. All maintenance operations were performed uniformly over the entire trial area.

Table G-1. Starting Seed for Phenotypic, Agronomic and Environmental Interaction Assessment

Material[1]	Material Type	Phenotype	Monsanto Seed Lot #	Sites[2]
MON 88302	Test	Glyphosate-tolerant	11225246	All sites
Ebony	Control	Conventional	11225243	All sites
Hyola 401	Reference	Conventional	10001850	MNCA, NDBI[3], SKME
46A65	Reference	Conventional	11220680	ABLE, MBBR, MNCA, MNPY[3], MNRO, NDBI[3], NDCL, SKRA, SKSA[3], SKWA
71-45 RR	Reference	Glyphosate-tolerant[4]	11225379	MBBR, MBMI[3], MNRO, NDBI[3], SKRA, SKSA[3]
In Vigor 5630	Reference	Glufosinate-tolerant[4]	11225748	MBMI[3], NDBI[3], SKWA
71-30 CL	Reference	Conventional	10001852	ABLE, IARL, MNPY[3], NDBO, SKSA[3]
71-40 CL	Reference	Conventional	11261966	SKRA
Hyola 357 Magnum	Reference	Glyphosate-tolerant[4]	10001727	IARL, MBBR, MNPY[3], NDBO
In Vigor 5550	Reference	Glufosinate-tolerant[4]	10001855	MNPY[3], NDCL, SKME
In Vigor 5020	Reference	Glufosinate-tolerant[4]	11225944	ABLE, MBNW, SKSA[3]
In Vigor 5030	Reference	Glufosinate-tolerant[4]	11225943	SKRA, SKRO
Python 1651H	Reference	Conventional	11225749	MBBR, NDBO, SKWA
Q2	Reference	Conventional	11220679	MBMI[3], MBNW, NDVA, SKRO, SKME
45H73	Reference	Conventional	10008476	MBMI[3], MBNW, NDCL, SKRO
Cropland 601	Reference	Conventional	10001849	IARL
In Vigor 8440	Reference	Glufosinate-tolerant[4]	11225747	IARL
50 Calibur	Reference	Glyphosate-tolerant[4]	11225744	NDCL
72-55 RR	Reference	Glyphosate-tolerant[4]	11225751	NDBO, SKME
HiLite 618	Reference	Conventional	10001851	MNRO, NDVA
52-41 RR	Reference	Glyphosate-tolerant[4]	11225752	NDVA, SKRO
45H28	Reference	Glyphosate-tolerant[4]	11225746	MNCA
30 Calibur	Reference	Glyphosate-tolerant[4]	11225745	MNCA
34-65 RR	Reference	Glyphosate-tolerant[4]	10001423	MNRO, SKWA
30-42 RR	Reference	Glufosinate-tolerant[4]	11225750	ABLE
45H26	Reference	Glyphosate-tolerant[4]	10008474	MBNW, NDVA

[1]MON 88302 and conventional control were planted at all field sites; the commercial reference varieties were site-specific

[2]Site codes are as follows: ABCA = Camrose, AB; ABFS = Sturgeon, AB; ABLE = Leduc, AB; IARL = Brookings, SD; MBBR = Elton, MB; MNCA = Otter Tail, MN; MBMI = Whitewater, MB; MBNW = Portage le Prairie, MB; MNPY = Stearns, MN; MNRO = Wilkins, MN; NDBI = Grand Forks, ND; NDBO = McHenry, ND; NDCL = Brookings, SD; NDGF = Grand Forks, ND; NDVA = McHenry, ND; SKME = Flett's Springs, SK; SKRA = Corman Park, SK; SKRO = Wallace, SK; SKSA = Rosthern, SK; SKWA = Viscount, SK.

[3]Sites with arthropod collections.

[4]Commercially available herbicide-tolerant canola varieties.

Table G-2. Field and Planting Information

Site[1]	Planting Date[2]	Planting rate (seeds/ft.^{2})	Planting depth (inches)	Plot area (ft.^{2}) ‡	Seeder passes/ plot	Soil series, organic matter, pH	Cropping History 2007	Cropping History 2008
ABLE	05/15/09	14.0	0.6	108	1	Morinville clay loam, 10%, 6.3	Wheat	Barley
IARL	05/15/09	14.0	1.0	114	1	Barnes clay loam,.3%, 7.1	Maize	Soybean
MBBR	05/21/09	18.0	0.4	85	1	Newdale clay loam, 5.5%, 7-8.4	Wheat	Wheat
MBMI	05/28/09	12.0	0.5	754	4	Ryerson clay loam, 5.4%, 7.3	Canola	Wheat
MBNW	05/26/09	18.0	0.4	85	1	Read Rome clay loam, 5.2%, 7.2-7.9	Dry Bean	Wheat
MNCA	05/30/09	12.0	0.5	130	1	Sisseton Heimdal loam, 3%, 7	Soybean	Wheat
MNPY	05/21/09	12.0	0.8	751	4	Estherville loam, 3.4%, 6.4	Maize	Fallow
MNRO	05/24/09	12.0	0.5	130	1	Aazdahl clay loam, 4-6%, 6.6-7.3	Soybean	Wheat
NDBI	05/22/09	14.0	0.8	665	4	Gardena silt loam, 4.5%, 6.8	Fallow	Wheat
NDBO	05/20/09	12.0	0.5	131	1	Svea-Barnes loam, 3.3%, 6.9	Flax	Wheat
NDCL	05/15/09	14.0	1.0	114	1	Barnes clay loam , 4.5%, 7.1	Soybean	Maize
NDVA	05/19/09	12.0	0.5	131	1	Williams loam, 2.4%, 5.4	Durum	Durum
SKME	05/28/09	15.0	0.8	106	1	Melfort clay loam, 9.1%, 6.5	Canola	Wheat
SKRA	05/17/09	15.0	0.8	106	1	Weyburn loam, 3.3%, 7.5	Fallow	Wheat
SKRO	05/19/09	15.0	0.5	103	1	Canora silty clay loam, 6.5%, 7.7	Wheat	Oats
SKSA	05/27/09	17.0	0.5	551	4	Hamlin loam , 2.19%, 6.35	Canola	Barley
SKWA	05/22/09	15.0	0.8	106	1	Weyburn loam, 4.6%, 6.7	Flax	Barley

[1]Site codes are as follows: ABCA = Camrose, AB; ABFS = Sturgeon, AB; ABLE = Leduc, AB; IARL = Brookings, SD; MBBR = Elton, MB; MNCA = Otter Tail, MN; MBMI = Whitewater, MB; MBNW = Portage le Prairie, MB; MNPY = Stearns, MN; MNRO = Wilkins, MN; NDBI = Grand Forks, ND; NDBO = McHenry, ND; NDCL = Brookings, SD; NDGF = Grand Forks, ND; NDVA = McHenry, ND; SKME = Flett's Springs, SK; SKRA = Corman Park, SK; SKRO = Wallace, SK; SKSA = Rosthern, SK; SKWA = Viscount, SK.
[2]Month-day-year (MM/DD/YY).
‡Sites with arthropod collection (MBMI, MNPY, NDBI and SKSA) had larger plot areas.

G.5. Phenotypic Observations

The description of the characteristics measured and the designated developmental stages where observations occurred are listed in Table VII-1.

G.6. Environmental Interaction Observations

Environmental interactions (*i.e.*, interactions between the crop plants and their receiving environment) were used to characterize MON 88302 by evaluating plant response to abiotic stress, disease damage, arthropod-related damage and pest and beneficial arthropod abundance in the plots using the methods described in sections G.7 and G.8.

G.7. Abiotic Stress Response, Disease Damage, and Arthropod-Related Damage

Qualitative Assessments: Abiotic Stress Response, Disease Damage and Arthropod Damage

MON 88302 and the conventional control were evaluated at all 17 sites for differences in plant response to abiotic stressors, disease damage and arthropod-related damage. Three abiotic stressors, three diseases and three arthropod pests were evaluated four times during the growing season at the following intervals:

Observation 1: Seedling to rosette stage
Observation 2: Bud to first flowering stage
Observation 3: Full flowering to flower completion stage
Observation 4: Pod development stage

The principal investigator at each site chose abiotic stressors, diseases and arthropod pests that were either actively causing plant injury in the study area or were likely to occur in canola during a given observation period. Therefore, abiotic stressors, diseases and arthropod pests assessed often varied between observations at a site and between sites.

Abiotic stressor and disease damage observations were collected from a single seeder pass of each plot (sites without arthropod collection) and from seeder pass 2 of each plot (sites with arthropod collection) using a continuous 0 – 9 scale of increasing severity. Data were collected numerically and then placed into one of the following categories for reporting purposes:

Rating	Severity of plant damage
0	none (no symptoms observed)
1 – 3	slight (symptoms not damaging to plant development)
4 – 6	moderate (intermediate between slight and severe)
7 – 9	severe (symptoms damaging to plant development)

Quantitative Assessments: Flea Beetle and Seedpod Weevil Damage Assessment

Flea beetle and seedpod weevil damage were evaluated quantitatively at the MBMI, MNPY, NDBI and SKSA sites. Flea beetle damage was assessed 1-2 times during growing season. The first assessment was conducted during two to three weeks following emergence. The second assessment was conducted approximately 1 week later. At each assessment time, flea beetle damage was assessed by examining 10 non-systematically selected plants from the middle of seeder pass 3 of each plot using a 0 - 10 rating scale adapted from (Palaniswamy and Lamb, 1992) where 0 = no damage and 10 = 100% of leaf area damaged.

For seedpod weevil damage assessment, pods were sampled from 10 non-systematically selected plants (five pods per plant) from the middle of seeder pass 3 of each plot at the ripening stage. Seedpod weevil damage was assessed by counting the number of exit holes in each pod.

G.8. Arthropod Abundance

Pest and beneficial arthropods were collected at the MBMI, MNPY, NDBI and SKSA sites four times during the growing season at the following time intervals:

Collection 1: Bud to first flowering stage
Collection 2: Approximately 2 weeks after collection 1
Collection 3: Approximately 2 weeks after collection 2
Collection 4: Approximately 2 weeks after collection 3

Arthropods were collected using a cone beat sheet sampling method. The cone beat sheet consists of a white sheet approximately 91 cm long, 46 cm wide at the top and 10 cm wide at the base, to which a container lid has been attached. The attached container lid has a hole in the middle to allow arthropods to pass through. Plants were shaken vigorously into a cone beat sheet (larger plants were folded in half), to collect the dislodged insects into the container. Arthropods were collected from five non-systematically selected plants from the middle of seeder pass 3 of each plot. The arthropods from all plants were combined into one pre-labeled container and placed on frozen ice packs. The samples collected from U.S. field sites and Canadian field sites were then sent overnight to Monsanto Company, St. Louis, MO and Department of Agricultural, Food and Nutritional Science at University of Alberta, respectively for arthropod identification and enumeration.

A maximum of the five most abundant pest and five most abundant beneficial arthropods were determined from four non-systematically selected samples for each collection from each individual site (*e.g.,* Collection 1, MBMI site). These specific arthropods were then enumerated across all samples (*i.e.*, one sample per plot) from a given collection at each individual site. The arthropods assessed often varied between collections from a site and between sites due to differences in temporal activity and geographical distribution of the taxa.

G.9. Environmental Interactions Evaluation Criteria

For the qualitative assessments of abiotic stress response, disease damage, and arthropod damage MON 88302 and the conventional control were considered different in susceptibility or tolerance to an abiotic stressor, disease or arthropod pest on a particular observation date if the range of injury severity to MON 88302 did not overlap with the range of injury severity to the conventional control across all four replicates. These data are categorical and were not subjected to statistical analysis. For each observation at a site, the range of injury severity across the commercial reference varieties provided a range of comparative values that are representative of commercially-released canola varieties. Arthropod abundance, flea beetle damage and seedpod weevil damage data were quantitatively evaluated and subjected to statistical analysis (Section G.11.).

G.10. Data Assessment

Experienced scientists familiar with the experimental design and evaluation criteria were involved in all components of data collection, summarization, and analysis. Study personnel assessed that measurements were taken properly, data were consistent with expectations based on experience with the crop, and the experiment was carefully monitored. Prior to analysis, the overall dataset was evaluated for evidence of biologically relevant changes and for possible evidence of an unexpected plant response. Any unexpected observations or issues during the study that would impact the study objectives were noted. Data were then subjected to statistical analysis as indicated below.

G.11. Statistical Analysis

Analysis of variance (ANOVA) was conducted according to a randomized complete block design using SAS (Version 9) to compare MON 88302 and the conventional control for the phenotypic characteristics listed in Table VII-1. Comparisons of MON 88302 and the conventional control were conducted within site (individual site analysis) and across sites (combined-site analysis). The level of statistical significance was predetermined to be 5% ($\alpha = 0.05$). MON 88302 and the conventional control were not statistically compared to the commercial reference varieties. Minimum and maximum mean values were calculated for each characteristic from the 24 unique commercial reference varieties that were included in this study. Data excluded from the study and the reasons for their exclusion are listed in Table G-3. Exclusion of these data did not adversely affect the quality of the study.

An analysis of variance (ANOVA) was conducted according to a randomized complete block design using SAS (Version 9) for the flea beetle damage, seedpod weevil damage and the arthropod abundance. The level of statistical significance was predetermined to be 5% ($\alpha = 0.05$). MON 88302 was compared to the conventional control at each site (individual site analysis) for flea beetle damage, seedpod weevil damage and arthropod abundance. Additionally, flea beetle damage and seedpod weevil damage data were pooled across sites (combined-site analysis) for a statistical comparison between MON 88302 and the conventional control. Minimum and maximum mean values were

calculated for flea beetle damage and seedpod weevil damage from 10 commercial reference varieties that were included at the MBMI, MNPY, NDBI and SKSA sites. The reference range for the arthropod abundance evaluated from a given collection and site was determined from the minimum and maximum damage values collected from the commercial reference varieties at the site. Data excluded from the study and the reasons for their exclusion are listed in G-3. Exclusion of these data did not adversely affect the quality of the study.

G.12. Individual Field Site Plant Growth, Development and Environmental Interactions Results and Discussion

In the individual site analysis, a total of 45 statistically significant differences were detected out of 148 comparisons between MON 88302 and the conventional control (Table G-4). These differences were distributed among all 12 of the phenotypic characteristics. Early stand count was lower for MON 88302 than the conventional control at the SKME site (23.6 vs. 29.0 plants/linear meter). MON 88302 was less vigorous (*i.e.*, higher rating indicates less vigor) than the conventional control at MBMI (4.0 vs. 3.0 rating), MNPY (6.0 vs. 5.0 rating) and NDBO (5.3 vs. 3.5 rating). MON 88302 reached days to first flowering later than the conventional control at all sites with the exception of the SKRA site where it reached first flowering in the same number of days. MON 88302 took longer to reach seed maturity than the conventional control at the SKSA site (106.5 vs. 99.5 days). MON 88302 had less lodging than the conventional control at the MNRO site (1.0 vs. 1.5 rating), but had more lodging than the conventional control at the NDBI site (3.3 vs. 2.0). MON 88302 was taller than the conventional control at the MNCA site (51.3 vs. 43.5 inches), MNRO (55.8 vs. 52.3 inches), NDBI (55.8 vs. 51.8 inches) and the SKME (43.3 vs. 40.8 inches) sites. However, MON 88302 was shorter than the conventional control at the IARL site (41.8 vs. 44.0 inches). MON 88302 had less visual pod shattering than the conventional control at the MNCA site (4.0 vs. 4.8 rating). MON 88302 had lower quantitative pod shattering than the conventional control at the MBMI site (155.9 vs. 365.0 seeds/ft.^{2}). MON 88302 had higher seed moisture than the conventional control at all sites except ABLE, NDBI, SKRA and SKWA. MON 88302 had lower seed quality (*i.e.*, more green seed) than the conventional control at IARL (6.5% vs. 1.8%), MNPY (0.5% vs. 0.0%), and MNRO (11.3% vs. 6.8%). MON 88302 had a higher yield than the conventional control at the MNCA (19.4 vs. 15.5 bu/ac) and MNRO (50.1 vs. 43.6 bu/ac) sites. MON 88302 had a lower final stand count than the conventional control at the MBNW site (13.6 vs. 19.8 plants/linear meter).

The statistical differences detected in the individual site analyses for early stand count, seedling vigor, seed maturity, lodging, plant height, visual pod shattering, quantitative pod shattering, seed quality, yield and final stand counts were not detected in the combined-site analysis. Thus, the differences detected for these phenotypic characteristics are not indicative of a consistent response associated with the trait and are unlikely to be biologically meaningful in terms of increased weediness potential of MON 88302 compared to the conventional control (Figure VII-1, step 2, “no” answer).

While a statistical difference was detected for days to first flowering and seed moisture in both the individual site analysis and the combined-site analysis, the mean values of MON 88302 for days to first flowering and seed moisture in the combined-site analysis were within the range of values for commercial reference varieties (Figure VII-1, step 3, "no" answer). Therefore, the difference in days to first flowering and seed moisture are unlikely to be biologically meaningful in terms of increased weediness potential.

In the individual site analysis of arthropod damage among all observations at the four sites, one statistically significant difference was detected out of 9 comparisons between MON 88302 and the conventional control (Table G-8). Lack of variability in the data precluded statistical comparisons between MON 88302 and the conventional control for two additional comparisons involving seedpod weevil; however, the means for MON 88302 and the conventional control were the same value for these comparisons, indicating no biological differences. MON 88302 had more flea beetle damage than the conventional control during Observation 1 at the MBMI site (1.18 vs. 0.53 rating). The statistical difference detected for flea beetle damage was not detected when the data were pooled across all four sites. Thus, this difference was not indicative of a consistent plant response associated with the trait and is unlikely to be biologically meaningful in terms of an adverse environmental impact of MON 88302 compared to conventional canola (Figure VII-1, step 2, "no" answer).

Table G-3 Data Missing or Excluded from Analysis

Site[1]	Substance	Rep	Characteristic	Reason for exclusion
ABLE	46A65	3	Seed maturity	Protocol revision to increase the precision of this assessment.
ABLE	MON 88302	1 & 2	Seed maturity	Protocol revision to increase the precision of this assessment.
IARL	All	All	Days to first flowering	Data was not recorded correctly.
IARL	All	All	Visual pod shattering	Data assessment added to the protocol by amendment after harvest was completed.
IARL	All	All	Abiotic stressors, disease and arthropod damage ratings	Data not recorded.
MBBR	All	All	Seed maturity, lodging, plant height, visual pod shattering, seed moisture, seed quality, yield and final stand count	A hail storm destroyed the plants after flowering started.
MBBR	All	All	Seedling vigor	Data were not recorded or was recorded incorrectly.
MBBR	Ebony	2	All	The results of the seeding quality testing indicated that one control plot may have contained plants of MON 88302.
MBNW	All	All	Seedling vigor	Data were not recorded or were recorded incorrectly.
MNCA	All	All	Seedling vigor	Data were not recorded or were recorded incorrectly.
MNCA	All	All	Abiotic stressors and disease damage ratings	Data was not recorded correctly.
MNPY	All	All	Plant height, abiotic stressors ratings	Data was not recorded correctly.
MNRO	All	All	Seedling vigor	Data were not recorded or were recorded incorrectly.
MNRO	All	All	Abiotic stressors and disease damage ratings	Data was not recorded correctly.
NDBO	All	All	Seed maturity	Data was not recorded correctly.
NDBO	MON 88302	1 & 4	Seed moisture	Instrument failure.
NDCL	ALL	ALL	Visual pod shattering	Data assessment added to the protocol by amendment after harvest was completed.
NDCL	ALL	ALL	Abiotic stressors, disease and arthropod damage ratings	Data not recorded.

Table G-3. Data Missing or Excluded from Analysis (continued)

Site[1]	Substance	Rep	Characteristic	Reason for exclusion
NDCL	All	All	Days to first flowering	Data was not recorded correctly.
NDCL	MON 88302 & Ebony	1	Seed maturity	Protocol revision to increase the precision of this assessment.
NDVA	All	All	Seed maturity	Data was not recorded correctly.
NDVA	Q2	All	Seed moisture	Instrument failure.
NDVA	HiLite 618	1 & 3	Seed moisture	Instrument failure.
NDVA	45H26	4	Seed moisture	Instrument failure.
NDVA	MON 88302	4	Seed moisture	Instrument failure.
SKME	All	All	Seed maturity	Data was not recorded correctly.
SKRA	MON 88302	3 & 4	Days to first flowering	Data not recorded.
SKRA	All	All	Seed maturity	Data was not recorded correctly.
SKRA	All	All	Abiotic stressors, disease and arthropod damage ratings	First two of the four total ratings were not recorded.
SKSA	All	All	Seedling vigor and seed moisture	Data was not recorded correctly.
SKSA	All	All	Quantitative pod shattering	Snow storm prevented the collection of this data.
SKSA	All	All	First arthropod collection and second flea beetle assessment	Weather prevented the collection of arthropods and assessment of flea beetle damage.
SKWA	All	All	Seed maturity	Data was not recorded correctly.
SKWA	All	All	Abiotic stressors, disease and arthropod damage	First of four ratings was not recorded.

[1]Site codes are as follows: ABCA=Camrose, AB; ABFS=Sturgeon, AB; ABLE=Leduc, AB; IARL=Brookings, SD; MBBR=Elton, MB; MNCA=Otter Tail, MN; MBMI=Whitewater, MB; MBNW=Portage le Prairie, MB; MNPY=Stearns, MN; MNRO=Wilkins, MN; NDBI=Grand Forks, ND; NDBO=McHenry, ND; NDCL=Brookings, SD; NDGF=Grand Forks, ND; NDVA=McHenry, ND; SKME=Flett's Springs, SK; SKRA=Corman Park, SK; SKRO=Wallace, SK; SKSA=Rosthern, SK; SKWA=Viscount, SK.

Table G-4. Individual Site Phenotypic Comparison of MON 88302 to Conventional Control

	Phenotypic Characteristic (units)					
	Early stand count[2] (number of plants per linear meter)		Seedling vigor[3] (1-9 scale)		Days to first flowering[4] (days after planting)	
	MON 88302	Control	MON 88302	Control	MON 88302	Control
Site [1]	Mean (S.E.)	Mean (S.E.)	Mean (S.E.)	Mean (S.E.)	Mean (S.E.)	Mean (S.E.)
ABLE	12.8 (1.9)	18.3 (1.6)	6.3 (0.5)	5.5 (0.3)	61.0 (0.0) *	59.0 (0.0)
IARL	21.7 (1.9)	21.5 (2.4)	6.0 (0.0)	6.0 (0.0)	–	–
MBBR	7.1 (1.1)	6.8 (0.6)	–	–	68.0 (0.0) *	63.0 (0.0)
MBMI	17.8 (1.4)	19.1 (0.6)	4.0 (0.0)*	3.0 (0.0)	57.0 (0.0)*	51.0 (0.0)
MBNW	14.8 (1.6)	20.1 (1.7)	–	–	62.0 (0.0) *	54.3 (0.8)
MNCA	23.7 (1.2)	20.8 (2.2)	–	–	63.3 (0.8)*	57.0 (0.0)
MNPY	26.8 (1.1)	24.5 (0.5)	6.0 (0.0)*	5.0 (0.0)	100.0 (0.0)*	94.0 (0.0)
MNRO	22.6 (0.8)	20.2 (0.9)	–	–	52.0 (0.0) *	47.5 (0.5)
NDBI	19.9 (1.5)	17.9 (1.7)	7.0 (0.0)	6.3 (0.5)	58.3 (0.5)*	50.5 (0.9)
NDBO	17.8 (2.1)	16.6 (0.8)	5.3 (0.8)*	3.5 (0.3)	53.0 (0.0)*	48.5 (0.6)
NDCL	23.0 (0.5)	24.9 (0.5)	7.0 (0.0) †	6.0 (0.0)	–	–
NDVA	14.3 (1.3)	13.2 (1.5)	6.5 (0.5)	5.8 (0.6)	54.0 (0.0)*	49.0 (0.0)
SKME	23.6 (1.2)*	29.0 (1.7)	3.3 (0.5)	3.3 (0.3)	56.0 (0.0)*	53.0 (0.0)
SKRA	22.0 (0.9)	21.8 (1.9)	3.3 (0.3)	3.0 (0.0)	54.0 (0.0)	54.0 (0.0)
SKRO	8.8 (1.9)	10.6 (1.9)	3.0 (0.0)	3.5 (0.3)	59.0 (0.0)*	57.8 (0.5)
SKSA	10.5 (1.1)	14.3 (0.8)	–	–	53.8 (0.3)*	50.5 (0.3)
SKWA	23.0 (3.2)	23.3 (2.3)	3.5 (0.3)	3.0 (0.0)	61.0 (0.0)*	56.0 (0.0)

Table G-4. Individual Site Phenotypic Comparison of MON 88302 to Conventional Control (continued)

	Phenotypic Characteristic (units)					
	Seed maturity[5] (days after planting)		Lodging[6] (1 – 9 scale)		Plant height[7] (inches)	
	MON 88302	Control	MON 88302	Control	MON 88302	Control
Site[1]	Mean (S.E.)	Mean (S.E.)	Mean (S.E.)	Mean (S.E.)	Mean (S.E.)	Mean (S.E.)
ABLE	108.0 (0.0)†	108.0 (0.0)	1.0 (0.0)†	1.0 (0.0)	36.3 (0.8)	36.0 (0.4)
IARL	90.3 (1.4)	88.8 (0.8)	1.0 (0.0)†	1.0 (0.0)	41.8 (1.0)*	44.0 (1.2)
MBBR	–	–	–	–	–	–
MBMI	101.0 (0.0)†	101.0 (0.0)	1.0 (0.0)	2.0 (0.0)	52.5 (1.3)	53.0 (0.8)
MBNW	105.0 (0.0)	104.0 (1.0)	1.0 (0.0)	1.8 (0.5)	54.5 (0.9)	51.5 (2.7)
MNCA	101.0 (0.0)†	101.0 (0.0)	1.0 (0.0)†	1.0 (0.0)	51.3 (0.5)*	43.5 (0.5)
MNPY	118.0 (0.0)	118.0 (0.0)	9.0 (0.0)†	9.0 (0.0)	–	–
MNRO	91.0 (0.0)†	86.0 (0.0)	1.0 (0.0)*	1.5 (0.3)	55.8 (0.5)*	52.3 (1.9)
NDBI	104.0 (0.0)	101.8 (0.8)	3.3 (0.5)*	2.0 (0.4)	55.8 (1.8)*	51.8 (0.9)
NDBO	–	–	1.0 (0.0)	1.0 (0.0)	42.8 (0.9)	42.5 (1.2)
NDCL	92.3 (1.7)	92.3 (1.7)	1.0 (0.0)	1.5 (0.3)	36.3 (2.2)	36.3 (2.3)
NDVA	–	–	1.3 (0.3)	1.0 (0.0)	45.0 (0.6)	44.8 (0.9)
SKME	–	–	1.0 (0.0)	1.5 (0.3)	43.3 (0.5)*	40.8 (0.8)
SKRA	–	–	1.0 (0.0)†	1.0 (0.0)	38.0 (0.8)	38.5 (0.9)
SKRO	112.0 (0.0)†	112.0 (0.0)	1.0 (0.0)	1.0 (0.0)	39.0 (0.6)	41.0 (1.6)
SKSA	106.5 (2.3)*	99.5 (1.9)	2.3 (0.3)	1.8 (0.3)	42.5 (0.5)	41.0 (0.7)
SKWA	–	–	1.0 (0.0)†	1.0 (0.0)	47.0 (1.5)	47.8 (0.3)

Table G-4. Individual Site Phenotypic Comparison of MON 88302 to Conventional Control (continued)

	Phenotypic Characteristic (units)					
	Visual pod shattering[8] (0-9 scale)		Quantitative pod shattering[9] (seeds per ft.^{2})		Seed moisture[10] (%)	
	MON 88302	Control	MON 88302	Control	MON 88302	Control
Site[1]	Mean (S.E.)	Mean (S.E.)	Mean (S.E.)	Mean (S.E.)	Mean (S.E.)	Mean (S.E.)
ABLE	1.0 (0.0)†	1.0 (0.0)	–	–	8.6 (0.1)	8.4 (0.1)
IARL	–	–	–	–	14.9 (0.6)*	12.1 (0.4)
MBBR	–	–	–	–	–	–
MBMI	1.0 (0.0)	1.0 (0.0)	155.9 (38.6)*	365.0 (53.7)	14.5 (0.4)*	12.8 (0.2)
MBNW	1.0 (0.0)	1.0 (0.0)	–	–	11.8 (0.4)*	9.9 (0.1)
MNCA	4.0 (0.4)*	4.8 (0.5)	–	–	13.2 (0.2)*	11.9 (0.1)
MNPY	1.0 (0.0)†	1.0 (0.0)	18.4 (0.8)	12.6 (0.9)	16.0 (0.4)*	15.2 (0.2)
MNRO	1.3 (0.3)	1.8 (0.3)	–	–	18.1 (0.1)*	15.4 (0.1)
NDBI	1.0 (0.0)	1.0 (0.0)	19.9 (4.8)	20.0 (6.5)	10.3 (0.2)	10.2 (0.1)
NDBO	1.0 (0.0)	1.0 (0.0)	–	–	24.2 (0.7)*	17.6 (1.6)
NDCL	–	–	–	–	9.1 (0.4)*	7.7 (0.0)
NDVA	1.0 (0.0)	1.5 (0.3)	–	–	14.4 (0.4)*	13.4 (0.4)
SKME	1.0 (0.0)†	1.0 (0.0)	–	–	13.0 (0.4)*	10.2 (0.2)
SKRA	1.0 (0.0)†	1.0 (0.0)	–	–	7.5 (0.2)	7.1 (0.2)
SKRO	1.0 (0.0)†	1.0 (0.0)	–	–	15.1 (0.5)*	12.5 (0.6)
SKSA	1.0 (0.0)	1.0 (0.0)	–	–	–	–
SKWA	1.0 (0.0)†	1.0 (0.0)	–	–	12.7 (0.2)	11.9 (0.3)

Table G-4. Individual Site Phenotypic Comparison of MON 88302 to Conventional Control (continued)

	Phenotypic Characteristic (units)					
	Seed quality[11] (%)		Yield[12] (bu per ac)		Final stand count[13] (number of stubs per linear meter)	
	MON 88302	Control	MON 88302	Control	MON 88302	Control
Site[1]	Mean (S.E.)	Mean (S.E.)	Mean (S.E.)	Mean (S.E.)	Mean (S.E.)	Mean (S.E.)
ABLE	0.8 (0.5)	0.5 (0.3)	29.5 (1.2)	26.6 (1.3)	13.8 (2.2)	15.3 (0.5)
IARL	6.5 (1.6)*	1.8 (0.6)	21.0 (1.8)	26.9 (2.4)	21.7 (1.9)	21.5 (2.4)
MBBR	–	–	–	–	–	–
MBMI	1.0 (1.0)	3.3 (1.7)	62.1 (3.5)	66.9 (2.3)	17.8 (1.4)	19.0 (0.7)
MBNW	0.5 (0.3)	1.5 (0.6)	72.3 (4.9)	66.2 (4.0)	13.6 (1.1)*	19.8 (1.6)
MNCA	3.3 (0.9)	3.0 (0.4)	19.4 (0.4)*	15.5 (0.7)	19.3 (0.6)	17.7 (2.3)
MNPY	0.5 (0.3)*	0.0 (0.0)	24.7 (0.2)	23.8 (0.3)	26.7 (1.0)	24.5 (0.5)
MNRO	11.3 (0.9)*	6.8 (1.4)	50.1 (1.2)*	43.6 (1.0)	20.6 (0.8)	18.6 (0.6)
NDBI	0.3 (0.3)	0.3 (0.3)	64.5 (3.2)	67.9 (4.7)	19.1 (2.6)	16.2 (1.9)
NDBO	8.5 (3.1)	4.5 (1.0)	29.6 (1.5)	35.1 (1.0)	17.4 (1.5)	16.2 (1.5)

Table G-4. Individual Site Phenotypic Comparison of MON 88302 to Conventional Control (continued)

	Phenotypic Characteristic (units)					
	Seed quality[11] (%)		Yield[12] (bu per ac)		Final stand count[13] (number of stubs per linear meter)	
	MON 88302	Control	MON 88302	Control	MON 88302	Control
Site [1]	Mean (S.E.)	Mean (S.E.)	Mean (S.E.)	Mean (S.E.)	Mean (S.E.)	Mean (S.E.)
NDCL	3.5 (0.6)	7.5 (3.1)	23.4 (2.1)	30.3 (3.5)	23.0 (0.5)	24.9 (0.5)
NDVA	9.3 (0.9)	10.3 (2.1)	40.4 (2.0)	38.4 (2.8)	16.7 (1.0)	13.8 (1.7)
SKME	1.3 (0.5)	0.0 (0.0)	45.3 (2.0)	52.7 (1.6)	20.9 (1.5)	21.3 (0.9)
SKRA	0.5 (0.3)	0.8 (0.5)	47.4 (1.5)	47.7 (2.1)	22.4 (1.3)	21.3 (2.0)
SKRO	3.8 (0.8)	2.5 (0.5)	58.1 (4.3)	66.6 (4.8)	9.1 (2.0)	9.9 (2.0)
SKSA	0.5 (0.3)	0.0 (0.0)	39.4 (1.0)	41.2 (3.6)	10.5 (0.9)	12.0 (0.5)
SKWA	1.0 (0.4)	2.5 (1.6)	60.3 (2.1)	62.6 (0.9)	19.7 (0.7)	17.7 (1.0)

Note: The experimental design was a randomized complete block with four replicates. S.E. = Standard Error. The number of plots (n) used in the statistical analysis was 4 except where noted..

(–) = Data not collected or excluded.

***** Indicates a statistically significant difference between MON 88302 and the conventional canola control ($\alpha = 0.05$).

† No statistical comparisons were made due to lack of variability in the data.

‡ Data not planned for collection at these sites.

[1] Site codes are as follows: ABCA=Camrose, AB; ABFS=Sturgeon, AB; ABLE=Leduc, AB; IARL=Brookings, SD; MBBR=Elton, MB; MNCA=Otter Tail, MN; MBMI=Whitewater, MB; MBNW=Portage le Prairie, MB; MNPY=Stearns, MN; MNRO=Wilkins, MN; NDBI=Grand Forks, ND; NDBO=McHenry, ND; NDCL=Brookings, SD; NDGF=Grand Forks, ND; NDVA=McHenry, ND; SKME=Flett's Springs, SK; SKRA=Corman Park, SK; SKRO=Wallace, SK; SKSA=Rosthern, SK; SKWA=Viscount, SK.

[2]Early stand counts were conducted by counting the number of plants from three separate linear meter rows data. At MBBR plot number (n)=4 for MON 88302 and N=3 for the conventional control.

[3]Rated on a scale of 1-9, where 1 =excellent vigor and 9 = poor vigor.

[4]Days to first flowering was determined from the number of days after planting when 50% of the plants in a plot had one or more flowers. Days to first flowering data were at IARL and NDCL plot number (n)=2 for MON 88302 and n=4 for the conventional control and at MBBR the plot number (n)=4 for MON 88302 and n=3 for the conventional control.

[5]Seed maturity was determined as the number of days after planting when 30% or more of the seed in the lower third of the main raceme had changed from a green to black/brown/tan color. Seed maturity data at ABLE plot number (n)=2 for MON 88302 and n=4 for the conventional control, at NDCL plot number (n)=3 for MON 88302 and the conventional control.
[6]Rated on a 0 - 9 scale, where 0 = completely upright plants and 9 = completely flat.
[7]Plant heights were measured from the soil surface to the top of the main raceme after flowering was completed.
[8]Visual pod shattering was estimated with a rated on a 1 - 9 scale, where 1 = 0 to 10% shatter, and each subsequent value on the scale increasing in 10% increments up to 9 = greater than 80% shatter.
[9]Collection trays placed within the crop canopy and seed losses from shattering counted once per week for three weeks. This data was intended for collection at MBMI, MNPY, NDBI and SKSA.
[10]Seed moisture data at NDBO plot number (n)=2 for MON 88302 and n=4 for the conventional control and at NDVA plot number (n)=3 for MON 88302 and n=4 for the conventional control.
[11]Seed quality was determined at harvest by counting the percentage of green seeds from a 100 seed subsample from each plot.
[12]Yield data at NDBO plot number (n)=2 for MON 88302 and n=4 for the conventional control at NDVA plot number (n)=3 for MON 88302 and n=4 for the conventional control.
[13]Final stand counts were conducted after harvest by measuring plant stems from three separate linear meter rows.

Table G-5. Abiotic Stress Response Evaluations of MON 88302 and Conventional Control Using an Observational Severity Scale

Abiotic stressor	Number of observations across all sites	Number of observations where no differences were observed between MON 88302 and the control
Total	131	130
Cold	9	9
Compaction	4	4
Drought	34	34
Flood	3	3
Frost	13	12*
Hail	19	19
Heat	22	22
Nitrogen deficiency	4	4
Wind	23	23

*A single difference was observed between MON 88302 and the conventional control during Observation 1 at the MBBR site: Test = severe, Control = moderate, Reference range = slight-severe. Data were not subjected to statistical analysis.

Note: The experimental design was a randomized complete block with four replicates. Observational data were collected at four crop development stages: Observation 1: seedling to rosette stage; Observation 2: bud to first flowering stage; Observation 3: full flowering to flower completion stage; Observation 4: pod development stage

Table G-6. Disease Damage Evaluations of MON 88302 and Conventional Control Using an Observational Severity Scale

Disease	Number of observations across all sites	Number of observations where no differences were observed between MON 88302 and the control
Total	141	141
Alternaria	12	12
Aster yellow	10	10
Bacterial leaf spot	3	3
Black leg	39	39
Cercospera leaf spot	4	4
Clubroot	8	8
Downy mildew	11	11
Fusarium	10	10
Phytophthora	2	2
Powdery mildew	1	1
Root rot	1	1
Sclerotina	31	31
Seedling blight	2	2
Seedling disease complex	1	1
White mold	4	4
White rust	2	2

No differences were observed between MON 88302 and the conventional control during any observation for damage caused by any of the assessed diseases. Data were not subjected to statistical analysis.
Note: The experimental design was a randomized complete block with four replicates. Observational data were collected at four crop development stages: Observation 1: seedling to rosette stage; Observation 2: bud to first flowering stage; Observation 3: full flowering to flower completion stage; Observation 4: pod development stage.

Table G-7. Arthropod-Related Damage Evaluations of MON 88302 and Conventional Control Using an Observational Severity Scale

Arthropod	Number of observations across all sites	Number of observations where no differences were observed between MON 88302 and the conventional control
Total	165	165
Alfalfa loopers (*Autographa californica*)	2	2
Aphids (Aphididae)	18	18
Bertha armyworms (*Mamestra configurata*)	15	15
Blister beetles (*Spicauta* spp.)	15	15
Cabbage seedpod weevils (*Ceutorhynchus obstrictus*)	4	4
Cabbage worms[1] (Pieridae)	8	8
Cutworms (Noctuidae)	10	10
Diamondback moth larvae (*Plutella xylostella)*	24	24
Flea beetles (Chrysomelidae)	25	25
Grasshoppers (Acrididae)	24	24
Lygus bugs (Miridae)	15	15
Red turnip beetles (*Entomoscelis Americana)*	4	4
Wireworms (Elateridae)	1	1

No differences were observed between MON 88302 and the conventional control during any observation for damage caused by any of the assessed arthropods. Data were not subjected to statistical analysis.
Note: The experimental design was a randomized complete block with four replicates. Observational data were collected at four crop development stages: Observation 1: seedling to rosette stage; Observation 2: bud to first flowering stage; Observation 3: full flowering to flower completion stage; Observation 4: pod development stage.
[1] Includes cabbage white caterpillars

Table G-8. Individual Site Analysis: Quantitative Assessment of Flea Beetle and Seedpod Weevil Damage to MON 88302 Compared to the Conventional Control in 2009 Field Trials

Pest	Damage assessment	Site[1]	MON 88302	Control
Flea beetles[2] (Chrysomelidae)	Mean (S.E.) damage of 10 plants per plot (0-10 rating scale) – Observation 1	MBMI	1.18 (0.25)*	0.53 (0.06)
		MNPY	0.63 (0.06)	0.83 (0.12)
		NDBI	2.63 (0.18)	2.70 (0.07)
		SKSA	0.30 (0.07)	0.48 (0.09)
	Mean (S.E.) damage of 10 plants per plot (0-10 rating scale) – Observation 2	MBMI	0.43 (0.13)	0.40 (0.09)
		MNPY	1.43 (0.05)	1.48 (0.08)
		NDBI	1.80 (0.21)	1.80 (0.22)
		SKSA	—	—
Seedpod weevil[3] (*Ceutorhynchus obstrictus*)	Mean (S.E.) number of holes in pods from 10 plants per plot)	MBMI	0.00 (0.00)†	0.00 (0.00)
		MNPY	0.13 (0.03)	0.02 (0.00)
		NDBI	0.00 (0.00)‡	0.00 (0.00)
		SKSA	0.00 (0.00)†	0.00 (0.00)

*Indicates a significant difference between MON 88302 and the conventional control ($\alpha = 0.05$).
†No statistical comparisons were made due to lack of variability in the data.
‡Mean values reported are zeros based on rounding of values that were small.
Note: The experimental design was a randomized complete block with four replicates. S.E. = Standard Error
[1]Site codes are as follows: MBMI = Whitewater, MB; MNPY = Stearns, MN; NDBI = Grand Forks, ND; SKSA = Rosthern, SK.
[2]Damage assessments for flea beetle were conducted 1-2 times during growing season. The first assessment was conducted during two to three weeks following crop emergence at all four sites. The second assessment was conducted approximately 1 week later at the MBMI, MNPY and NDBI sites. The first flea beetle assessment at SKSA could not be conducted due to a severe rain storm.
[3]Damage assessments for seedpod weevil were conducted at the ripening stage.
A dash (—) indicates information not available.

Table G-9. Abundance of Pest Arthropods in Cone Beat Sheet Samples Collected from MON 88302, Conventional Control and the Commercial Reference Varieties

Col.	Site[1]	Pest Arthropod[2]								
		Aphids (Aphididae)			Bertha armyworms (*Mamestra configurata*)			Diamondback moth larvae (*Plutella xylostella*)		
		MON 88302 Mean (S.E.)	Control Mean (S.E.)	Reference Range[3]	MON 88302 Mean (S.E.)	Control Mean (S.E.)	Reference Range[3]	MON 88302 Mean (S.E.)	Control Mean (S.E.)	Reference Range[3]
1	MBMI	–	–	–	–	–	–	1.00 (0.00)	0.50 (0.29)	0.00 – 1.25
	MNPY	1.00 (1.00)	0.25 (0.25)	0.25 – 1.25	–	–	–	–	–	–
	NDBI	–	–	–	–	–	–	–	–	–
	SKSA	–	–	–	–	–	–	–	–	–
2	MBMI	–	–	–	–	–	–	0.25 (0.25)	0.50 (0.29)	0.00 – 0.25
	MNPY	0.50 (0.29)	1.50 (0.87)	2.75 – 4.50	–	–	–	–	–	–
	NDBI	0.00 (0.00)	0.25 (0.25)	0.00 – 0.25	–	–	–	–	–	–
	SKSA	–	–	–	–	–	–	–	–	–
3	MBMI	0.50 (0.50)	0.00 (0.00)	0.00 – 0.5	–	–	–	1.00 (0.58)	0.25 (0.25)	0.00 – 0.25
	MNPY	0.50 (0.29)	0.75 (0.75)	0.00 – 17.00	0.00 (0.00)	0.50 (0.29)	0.00 – 0.50	–	–	–
	NDBI	0.00 (0.00)	0.25 (0.25)	0.00 – 3.00	–	–	–	–	–	–
	SKSA	–	–	–	–	–	–	–	–	–
4	MBMI	2.75 (1.60)	2.50 (1.26)	0.75 – 9.25	–	–	–	2.50 (1.44)	3.00 (0.71)	0.75 – 1.25
	MNPY	0.25 (0.25)	0.25 (0.25)	0.00 – 0.25	–	–	–	0.25 (0.25)	0.25 (0.25)	0.00 – 0.25
	NDBI	–	–	–	–	–	–	–	–	–
	SKSA	–	–	–	–	–	–	0.00 (0.00)	0.75 (0.48)	0.00 – 0.50

Table G-9. Abundance of Pest Arthropods in Cone Beat Sheet Samples Collected from MON 88302, Conventional Control and the Commercial Reference Varieties (continued)

Col.	Site[1]	Pest Arthropod[2]								
		Flea beetles (Chrysomelidae)			Lygus bugs (Miridae)			Thrips (Thripidae)		
		MON 88302 Mean (S.E.)	Control Mean (S.E.)	Reference Range[3]	MON 88302 Mean (S.E.)	Control Mean (S.E.)	Reference Range[3]	MON 88302 Mean (S.E.)	Control Mean (S.E.)	Reference Range[3]
1	MBMI	–	–	–	–	–	–	0.50 (0.50)	0.25 (0.25)	0.00 – 0.50
	MNPY	–	–	–	0.00 (0.00)	0.00 (0.00)	0.00 – 0.25	–	–	–
	NDBI	0.00 (0.00)	0.00 (0.00)	0.00 – 0.75	–	–	–	1.75 (0.75)	0.25 (0.25)	4.00 – 13.50
	SKSA	–	–	–	–	–	–	–	–	–
2	MBMI	–	–	–	–	–	–	0.75 (0.75)	0.00 (0.00)	0.00 – 0.25
	MNPY	–	–	–	–	–	–	–	–	–
	NDBI	–	–	–	–	–	–	0.25 (0.25)	0.75 (0.75)	2.00 – 5.00
	SKSA	–	–	–	0.00 (0.00)	0.00 (0.00)	0.00 – 0.00	8.25 (5.12)	5.00 (0.58)	2.75 – 4.00
3	MBMI	0.25 (0.25)	0.00 (0.00)	0.00 – 0.25	–	–	–	4.25 (2.46)	0.25 (0.25)	0.00 – 0.75
	MNPY	–	–	–	0.25 (0.25)	1.25 (0.63)	0.50 – 2.25	1.00 (0.71)	0.00 (0.00)	0.00 – 0.25
	NDBI	0.50 (0.29)	2.00 (0.82)	0.00 – 0.75	–	–	–	4.00 (1.68)	2.75 (1.89)	0.50 – 2.75
	SKSA	–	–	–	–	–	–	0.50 (0.50)	3.25 (2.36)	0.00 – 3.50
4	MBMI	1.25 (0.95)	1.25 (0.48)	0.25 – 0.50	0.00 (0.00)	0.00 (0.00)	0.00 – 0.50	0.00 (0.00)	0.00 (0.00)	0.00 – 0.00
	MNPY	–	–	–	2.5 (0.50)	3.75 (0.85)	0.25 – 5.75	–	–	–
	NDBI	4.25 (0.25)	3.25 (2.36)	2.00 – 3.50	–	–	–	–	–	–
	SKSA	–	–	–	–	–	–	2.25 (1.32)	1.50 (1.19)	0.00 – 2.75

No statistically significant differences were detected between MON 88302 and the conventional control ($\alpha = 0.05$).
Note: The experimental design was a randomized complete block with four replicates. S.E. = Standard Error.
A dash (−) indicates arthropod not evaluated.
[1]Site codes are as follows: MBMI = Whitewater, MB; MNPY = Stearns, MN; NDBI = Grand Forks, ND; SKSA = Rosthern, SK.
[2]Arthropod collection 1 was made at bud to first flowering stage and the three subsequent collections at approximately two week intervals thereafter. The first arthropod collection could not be conducted at SKSA due to a severe rain storm.
[3]Reference range = minimum and maximum mean values among the commercial reference varieties.

Table G-10. Abundance of Beneficial Arthropods in Cone Beat Sheet Samples Collected from MON 88302, Conventional Control and the Commercial Reference Varieties

		Beneficial Arthropod[2]								
		Chironomid midge			Lacewings (chrysopidae)			Ladybird beetles (Coccinellidae)		
Col.	Site[1]	MON 88302 Mean (S.E.)	Control Mean (S.E.)	Reference Range[3]	MON 88302 Mean (S.E.)	Control Mean (S.E.)	Reference Range[3]	MON 88302 Mean (S.E.)	Control Mean (S.E.)	Reference Range[3]
1	MBMI	–	–	–	–	–	–	–	–	–
	MNPY	–	–	–	–	–	–	–	–	–
	NDBI	–	–	–	–	–	–	–	–	–
	SKSA	–	–	–	–	–	–	–	–	–
2	MBMI	–	–	–	–	–	–	–	–	–
	MNPY	–	–	–	–	–	–	–	–	–
	NDBI	–	–	–	–	–	–	–	–	–
	SKSA	–	–	–	–	–	–	0.00 (0.00)	0.00 (0.00)	0.00 – 0.00
3	MBMI	–	–	–	–	–	–	–	–	–
	MNPY	–	–	–	–	–	–	0.00 (0.00)	0.00 (0.00)	0.00 – 0.50
	NDBI	–	–	–	–	–	–	–	–	–
	SKSA	0.00 (0.00)	0.00 (0.00)	0.00 – 0.25	–	–	–	–	–	–
4	MBMI	–	–	–	0.00 (0.00)	0.00 (0.00)	0.00 – 0.25	–	–	–
	MNPY	–	–	–	–	–	–	–	–	–
	NDBI	–	–	–	–	–	–	–	–	–
	SKSA	–	–	–	–	–	–	–	–	–

Table G-10. Abundance of Beneficial Arthropods in Cone Beat Sheet Samples Collected from MON 88302, Conventional Control and the Commercial Reference Varieties (continued)

Col.	Site[1]	Beneficial Arthropod[2]								
		Micro-parasitic hymenoptera			Macro-parasitic hymenoptera			*Orius* spp.		
		MON 88302 Mean (S.E.)	Control Mean (S.E.)	Reference Range[3]	MON 88302 Mean (S.E.)	Control Mean (S.E.)	Reference Range[3]	MON 88302 Mean (S.E.)	Control Mean (S.E.)	Reference Range[3]
1	MBMI	–	–	–	–	–	–	–	–	–
	MNPY	–	–	–	0.00 (0.00)	0.00 (0.00)	0.00 – 0.25	–	–	–
	NDBI	–	–	–	–	–	–	–	–	–
	SKSA	–	–	–	–	–	–	–	–	–
2	MBMI	0.00 (0.00)	0.00 (0.00)	0.00 – 0.25	–	–	–	–	–	–
	MNPY	–	–	–	–	–	–	–	–	–
	NDBI	–	–	–	–	–	–	0.00 (0.00)	0.25 (0.25)	0.00 – 0.00
	SKSA	–	–	–	–	–	–	–	–	–
3	MBMI	0.75 (0.75)	0.25 (0.25)	0.00 – 0.50	–	–	–	–	–	–
	MNPY	–	–	–	–	–	–	2.00 (1.08)	1.75 (0.85)	0.50 – 2.25
	NDBI	0.25 (0.25)	0.00 (0.00)	0.00 – 0.00	–	–	–	–	–	–
	SKSA	0.00 (0.00)	0.00 (0.00)	0.00 – 0.00	–	–	–	–	–	–
4	MBMI	–	–	–	–	–	–	–	–	–
	MNPY	–	–	–	–	–	–	1.50 (1.19)	0.25 (0.25)	0.00 – 0.00
	NDBI	–	–	–	–	–	–	–	–	–
	SKSA	0.00 (0.00)	0.25 (0.25)	0.00 – 0.25	–	–	–	–	–	–

Table G-10. Abundance of Beneficial Arthropods in Cone Beat Sheet Samples Collected from MON 88302, Conventional Control and the Commercial Reference Varieties (continued)

Col.	Site[1]	Beneficial Arthropod[2]					
		Spiders (Araneae)			Sphecid wasps (Sphecidae)		
		MON 88302 Mean (S.E.)	Control Mean (S.E.)	Reference Range[3]	MON 88302 Mean (S.E.)	Control Mean (S.E.)	Reference Range[3]
1	MBMI	–	–	–	–	–	–
	MNPY	–	–	–	0.00 (0.00)	0.00 (0.00)	0.00 – 0.25
	NDBI	–	–	–	–	–	–
	SKSA	–	–	–	–	–	–
2	MBMI	–	–	–	–	–	–
	MNPY	–	–	–	–	–	–
	NDBI	–	–	–	–	–	–
	SKSA	–	–	–	–	–	–
3	MBMI	–	–	–	–	–	–
	MNPY	–	–	–	–	–	–
	NDBI	–	–	–	–	–	–
	SKSA	–	–	–	–	–	–
4	MBMI	–	–	–	–	–	–
	MNPY	–	–	–	–	–	–
	NDBI	–	–	–	–	–	–
	SKSA	0.00 (0.00)	0.00 (0.00)	0.00 – 0.25	–	–	–

No statistically significant differences were detected between MON 88302 and the conventional control ($\alpha = 0.05$).
Note: The experimental design was a randomized complete block with four replicates. S.E. = Standard Error.
A dash (−) indicates arthropod not evaluated.
[1]Site codes are as follows: MBMI = Whitewater, MB; MNPY = Stearns, MN; NDBI = Grand Forks, ND; SKSA = Rosthern, SK.
[2]Arthropod collection 1 was made at bud to first flowering stage and the three subsequent collections at approximately two week intervals thereafter. The first arthropod collection could not be conducted at SKSA due to a severe rain storm.
[3]Reference range = minimum and maximum mean values among the commercial reference varieties.

References for Appendix G

NDSU. 2005. Canola production field guide. A-1280, North Dakota State University Extension Service, Fargo, North Dakota.

Palaniswamy, P. and R.J. Lamb. 1992. Screening for antixenosis resistance to flea beetles, Phyllotreta cruciferae (Goeze) (Coleoptera: Chrysomelidae), in rapeseed and related crucifers. Canadian Entomologist 124: 895-906..

Appendix H: Materials and Methods for Pollen Morphology and Viability Assessment

H.1. Plant Production

MON 88302, a conventional control, and four commercial reference varieties were grown in pots in a growth chamber established at 21 °C day/18 °C night with a 16 h photoperiod (Table H-1). The plants were arranged in a randomized complete block design with five replicates with 1 plant of each entry per replicate. Prior to planting, the starting seed was treated with Helix XTra (insecticide 20.7% thiamethoxam and fungicides 1.25% difenoconazole, 0.40% mefenoxam, and 0.13% fludioxonil) at the recommended rate. Canola was seeded into 4" pots with Metro-Mix 200 containing 14 oz/yds^3 Micromax soil medium. Three seeds of MON 88302, the conventional control or commercial reference varieties were planted in each pot at an approximate depth of ~ 0.25" (~ 0.6 cm). The pots were thinned to one plant per pot when all of the plants had approximately one true leaf. Pots were placed in watering trays with capillary mats and irrigated as needed. Fertilizer was applied approximately weekly at a rate of 75 to 100 parts per million (Peter's 20-20-20). Plants were not treated with glyphosate herbicide.

H.2. Flower Collection

A total of three newly opened flowers were collected from each plant. Anthers from each flower were removed and transferred to a container labeled with the pot number and containing 400 µl of Alexander's stain solution (Alexander, 1980). The stain solution was prepared on the day anthers were collected with a final concentration of lactic acid of 0.15%. Each tube was gently agitated with a vortex for approximately 30 seconds to release the pollen to the stain solution and stored for at least 24 h at approximately 4 °C prior to preparation and analysis.

H.3. Pollen Sample Preparation

Pollen samples were prepared in a laboratory using microscope slides labeled with the pot number. The pot number identifier was cross-referenced with the study notebook to verify the original entry. A 20 µl aliquot of the pollen/stain solution was then added to the slide and covered with a cover slip.

H.4. Data Collection

Pollen samples were assessed for viability, pollen grain diameter and general morphology. Samples were viewed under an Olympus Provis BX51TRF light/fluorescence microscope equipped with an Olympus DP70 digital color camera. The microscope and camera were connected to a computer running Microsoft Windows 2000 Professional (©1981-1999, Microsoft Corp.) and installed with associated camera software [DP Controller v1.2.1.108 and DP Manager v1.2.1.107, respectively (© 2001-2003, Olympus Optical Co., Ltd.)] and imaging software [Image-Pro Plus v6.2.1.491 (© 1993-2007, Media Cybernetics, Inc.)].

H.4.1. Pollen Viability

When exposed to the stain solution, viable pollen grains had a round to oval shape and stained red to purple due to the presence of living cytoplasmic content. Non-viable pollen grains stained blue to green and appeared round to collapsed in shape, depending on the degree of hydration (Alexander, 1980). For each pollen sample, the number of viable and non-viable pollen grains was counted from a minimum of 75 pollen grains from a random field of view under the microscope. Dense clusters of pollen or pollen grains adhering to flower parts were not counted because they may not have absorbed the stain solution uniformly.

H.4.2. Pollen Diameter

Micrographs (400X resolution) of ten representative pollen grains from one sample per plant were taken and imported into the imaging software. The software was used to measure pollen grain diameter along two perpendicular axes for each selected pollen grain. The mean of the twenty diameter values was calculated for each pollen sample.

H.4.3. General Pollen Morphology

General pollen morphology of MON 88302, conventional control and commercial reference varieties was observed from the micrographs that were used for pollen diameter measurements.

H.5. Statistical Analysis

Analysis of variance (ANOVA) was conducted according to a randomized complete block design with five replicates using SAS (Version 9). The level of statistical significance was predetermined to be 5% ($\alpha = 0.05$). MON 88302 was compared to the conventional control for percent viable pollen and pollen grain diameter. MON 88302 was not statistically different when compared to the commercial reference varieties. A reference range for each measured characteristic was determined from the minimum and maximum mean values from among the four commercial reference varieties. General pollen morphology was qualitatively assessed and not subjected to statistical analysis.

Table H-1. Starting Seed for Pollen Morphology and Viability Assessment

Substance	Substance Type	Phenotype	Monsanto Lot #
MON 88302	Test	Glyphosate-tolerant	11263712
Ebony	Control	Conventional	11263709
Dekalb 71-30 CL	Reference	Conventional	10001852
InVigor 5020	Reference	Glufosinate-tolerant	11225944
DK71-45	Reference	Glyphosate-tolerant	11225379
46A65	Reference	Conventional	11220680

References for Appendix H

Alexander, M.P. 1980. A versatile stain for pollen fungi, yeast and bacteria. Stain Technology 55: 13-18.

Appendix I: Petitioner's Environmental Report

I.1. Summary

Since its launch in 1997, Roundup Ready canola has been an excellent weed management tool for growers offering both a simple and cost-effective solution for broad spectrum weed control. The current Roundup Ready canola product RT73 has restrictions in application rates and timing which make the ability to control some important annual and perennial weeds challenging. Because application of glyphosate is limited to the 6-leaf stage and earlier in current Roundup Ready canola, weed control can become a significant issue when weather conditions prevent herbicide treatment until after the 6-leaf stage. MON 88302 is a second generation glyphosate-tolerant canola product that allows for a wider period of application up to first flower instead of the 6-leaf growth stage and provides tolerance that allows for a glyphosate application rate similar to the rates for glyphosate-tolerant maize and soybeans. The proposed maximum glyphosate application rate on MON 88302 is twice the currently labeled maximum application rate for RT73 and offers improved control of difficult weeds such as dandelion and Canada thistle. Monsanto has submitted a request for a review by the U.S. EPA of an amended label for use of glyphosate on glyphosate-tolerant canola, which incorporates the higher application rates and later timing of application. Under the proposed expanded glyphosate use pattern with MON 88302, glyphosate residue levels fall within the U.S. EPA's existing tolerance of 20 parts per million (ppm) for canola. In accordance with U.S. FDA's consultation policy (57 FR 22984-23005), Monsanto has also submitted to the U.S. FDA a food and feed safety assessment and nutritional assessment summary for MON 88302.

This environmental report (ER), which has been prepared to support an anticipated APHIS environmental assessment, evaluates two alternatives: the "deregulation in whole" alternative and the "no action" alternative. Under the deregulation in whole alternative, MON 88302 and any progeny derived from crosses between MON 88302 and conventional *Brassica* species, and crosses of MON 88302 with other biotechnology-derived canola that has been deregulated would no longer be a regulated article under 7 CFR Part 340 and would be widely available for planting. Under the no action alternative, MON 88302 would remain a regulated article and plantings could be conducted under APHIS notification or permit. Interstate movements and field trials of MON 88302 have been conducted under permits issued or notifications acknowledged by APHIS since 2005.

U.S. Production of Canola: Canola is a low erucic acid type of oilseed rape that has been produced in the U.S. since the 1980s, and is used primarily for edible oil and meal for feed. Before the development of canola, oilseed rape was unsuited for food or feed use because of the high erucic acid and high glucosinolate content. In 2010, approximately 1.45 million acres of canola were planted in the U.S. The major U.S. canola production area is in North Dakota and northwestern Minnesota. This production area in the U.S. is in the southern part of the major North American canola production area, which is primarily in Canada. This area is planted exclusively with spring canola, a type that is planted in spring and harvested in fall. In 2010, North Dakota and Minnesota produced 92% of the 1.1 million-metric ton U.S. canola crop, with almost all of this in North Dakota. After the North Dakota/Minnesota area, the highest 2010 canola production was in Oklahoma (4% of U.S. production in 2010). The southern plains production area uses winter canola varieties, which are planted in the fall and harvested in late

spring or early summer. The remaining 4% of U.S. production is both winter and spring canola grown primarily in Idaho, Montana, Oregon, Colorado, Kansas and Washington. Winter canola has higher yield potential than spring canola, but can be planted only where the plants can survive the winter. Although *Brassica rapa* and *Brassica juncea* varieties are also available and can be used to produce canola quality oil, almost all U.S. canola production is from *Brassica napus* varieties. U.S. production of canola is far short of U.S. demand, and the shortfall is made up primarily by imports of oil, seed, and meal from Canada. Biotechnology-derived canola varieties have been commercially available in the U.S. since 1999. In 2008 approximately 95% of the North Dakota canola crop was produced using biotechnology-derived varieties containing a herbicide tolerance trait, with approximately 56% glyphosate tolerant and 39% glufosinate tolerant.

Canola Seed Production: Approximately 5,000 acres is needed for production of the U.S. canola seed crop. Most canola seed grown for sale for crop production for the North American market is produced in the summer in a relatively small geographic area in southern Alberta, Canada and the northwestern U.S. states of Washington, Oregon, Idaho, North Dakota and Colorado.

Specialty and Organic Canola Production: Specialty canola includes high erucic acid varieties used in industrial processes, organic, and varieties with improved fatty acid profiles including high oleic and high oleic, low linoleic varieties. Based on the most recent USDA Census of Agriculture, which was conducted in 2008, there were approximately 232 acres of organic canola grown in the U.S., all grown in four states, none of which are major canola production states. As noted above, the vast majority of canola currently produced in the U.S. contains a herbicide-tolerant trait.

Other Brassica Production: Because of potential for cross pollination between canola (*B. napus*) and related *Brassica* species, this ER describes other *Brassica* crop and seed production. Some of these crops are organic; however, the available information on acreage and areas of production does not differentiate areas of organic and non-organic production.

The primary *Brassica* vegetable crops in the U.S. are *B. napus* (rutabaga and Siberian kale), *Brassica oleracea* (cabbage, broccoli, cauliflower, Brussels sprouts, collard, kale, kohlrabi), *B. juncea* (red giant mustard, sawtooth mustard, and others) and *B. rapa* crops (Chinese cabbage, pak choi, choi sum, turnip, mizuna, mibuna, tat soi and others). While *Brassica* vegetable crops are grown throughout the U.S., they are harvested before they produce seed, and are therefore not able to cross pollinate with other *Brassica* species.

The majority of the *Brassica* vegetable seed production occurs in three distinct areas: the Willamette Valley in Oregon; Skagit County in western Washington; and the Columbia Basin in eastern Washington. To maintain the desired vegetable seed quality, these areas have strict regulations or prohibitions on growing canola and other oilseed rape crops. *Brassica* vegetable seed crops are also produced in California and Arizona, and broccoli seed is grown primarily in California and Arizona. According to the 2007 Census of Agriculture, canola was grown on 16 acres in California and was not grown in Arizona.

Brassica and related field crops grown for seed in addition to canola include high erucic acid or industrial oilseed rape, mustard seed and biofumigant mustard seed. High erucic acid oilseed rape is grown on very limited acres in Washington, Oregon, and Idaho. These states all regulate

where canola and oilseed rape can be grown, to prevent cross pollination. The great majority of mustard seed grown in the U.S. is yellow mustard (*Sinapsis alba*); only very small acreages of *Brassica* mustard is grown.

Agronomic Practices in Canola Including Weed Management: Canola production typically involves the extensive use of agronomic inputs and cultivation practices to maximize productivity and grower profitability. Weeds are one of the primary pests that limit productivity.

The major weeds in canola are wild mustard, Canada thistle and wild oat, with volunteer cereal, flixweed, wild buckwheat, perennial sowthistle, dandelion and quackgrass also contributing to yield loss. The majority of canola acres are planted with glyphosate- and glufosinate-tolerant varieties and application of in-crop herbicides plus tillage practices are used to control weeds. In combination with appropriate agronomic practices, herbicide-tolerant canola volunteers can be controlled in the subsequent rotational crop with a variety of herbicides.

While some of these weeds have developed resistance to other herbicides in canola production states, no glyphosate-resistant population of these weed species has been reported in the major U.S. canola production area in North Dakota/Minnesota. The three- to four-year rotations commonly used for canola (recommended primarily for disease control), the fact that other herbicide modes of action are used in the rotational crops and that most rotational crops do not have glyphosate-tolerant varieties, plus the option of glyphosate- or glufosinate-tolerant canola varieties all combine to minimize the potential for development of glyphosate resistant weeds.

Interactions between Canola and the Environment

Canola is grown in highly managed agricultural settings and can interact with the environment directly, via pollen movement to sexually compatible plants and potential hybridization/gene introgression, or via persistence of canola plants themselves in non-agricultural settings.

Cross Pollination Potential – Other Canola Crops: Canola is predominantely self-pollinating, although interplant (plants are touching one another) cross pollination can occur at a rate of approximately 30%. Most canola pollen (98%) travels less than 12 meters from its source. However, canola pollen dispersal due to wind and insects can occur over greater distances at low frequency.

Cross Pollination Potential – Brassica Vegetable Species: In areas where plants are allowed to flower, canola could cross pollinate with *B. napus* vegetables such as rutabaga and Siberian kale. Cross pollination with *B. rapa* and *B. oleracea* vegetables is also possible, but less likely to occur. However, *B. napus*, *B. rapa* and *B. oleracea* vegetables are not considered weedy, and are generally harvested prior to flowering minimizing cross pollination, hybridization and seed formation. Additionally, cross pollination between canola and vegetable seed crops, whether organic or non-organic, is expected to be non-existent or negligible given that there are strict controls on canola production in the major *Brassica* vegetable seed production areas (Oregon and Washington). In other *Brassica* vegetable seed production areas (California and Arizona), there is negligible, if any, canola production or canola seed production.

Cross Pollination Potential – Native Brassica: No native *Brassica* species have been identified in North America; therefore there is no potential for cross pollination to native species.

Cross Pollination Potential – Naturalized or Weedy Brassica[2]: There are several *Brassica* weed species in the U.S. and Canada, including several that are common in canola production areas; however, none of these are *B. napus*, and the potential for introgression between *B. napus* canola and all other species except *B. rapa* is expected to range from very low to extremely low. Cross pollination between canola and *B. rapa* is rare, and gene introgression has been documented in only one case to our knowledge; in that case the hybrid population declined rapidly and was gone in a few years.

Ruderal Canola: *Brassica napus* is regarded as an opportunistic species; that is, a species adapted to take advantage of temporary conditions such as disturbed areas. It is not considered to be a colonizing or invasive species capable of establishing in undisturbed natural ecosystems. Therefore, if canola seeds, which are very small, are lost from transport vehicles, canola may be found growing at the edges of roadways and other transportation routes. Multiple studies of roadside or ruderal canola have found that canola populations generally persist only for a year or two, and are usually found within a few feet of a roadway in disturbed soil not yet colonized by grasses and other more competitive plants.

Public Health, Worker Safety and Animal Health: The primary food use for canola is canola oil, which has well established heart health benefits. Canola meal is used as an animal feed source and the canola plant has limited use as a forage crop. Pesticide use on canola including glyphosate is regulated by the U.S. EPA, which registers specific uses after finding no unreasonable adverse effects to human health and the environment resulting from that use. Glyphosate has been registered for use in canola production since the introduction of the first glyphosate-tolerant product RT73 in 1999.

Animal, Plant and Microbial Communties: The affected environment for growing canola plants can generally be considered the agroecosystem (managed agricultural fields) plus areas extending beyond the intended plantings that might be affected by agricultural operations. Canola can interact with the environment directly, via pollen movement to sexually compatible plants or via persistence of canola plants themselves in non-agricultural settings.

Animals that consume the canola seed or plant, inhabit the fields, prey on the small animals inhabiting the field, or live in streams draining the canola field are part of the affected environment. Plants on adjacent land may be affected by fertilizer runoff, water runoff, and/or herbicide drift. Threatened or endangered animal or plant species that may inhabit the surrounding area are also a part of the affected environment. Significant variation in soil microbial populations within and among agricultural fields is also expected due to fertilization and cultivation.

Land Use: Acreage planted in canola in the U.S. has increased in recent years, and may continue to increase based on U.S. demand exceeding U.S. production. Recent increases in canola production have resulted primarily from canola replacing wheat in wheat monocultures or cropping systems in which wheat is the primary crop, and this trend may continue. While canola

[2] As defined by the USDA Plant Database. A naturalized plant is any non-native plant that can survive on its own, and includes both weeds and non-weeds.

has potential as a biodiesel crop, soybean oil is currently substantially less expensive than canola oil in the U.S., limiting that potential use for canola.

Surface Water and Groundwater Quality: Surface water may be impacted from canola production by pesticide and herbicide runoff from canola fields. Based on the states' water quality reports to the U.S. EPA, pesticides and herbicides are relatively minor contributors to impairment of surface water in the U.S. compared to sedimentation and turbidity. Additionally, researchers have found significant increases in conservation tillage with the introduction of herbicide-tolerant canola.

Economics: Canola was planted on approximately 1.45M acres in the U.S. producing 1.1 million metric tons or 1.9% of the world's 60.6 million metric tons of canola in the 2010 growing season with a value of approximately $487 million. In the same year the U.S. imported 0.7 million metric tons from Canada. Total global U.S. imports of canola oil continue to increase steadily from 0.5 million metric tons in 2000 to 1.2 million metric tons in 2010.

Environmental Consequences and Comparison of Alternatives: Table I-1 presents a summary of the potential impacts associated with selection of either of the alternatives evaluated in this ER.

Table I-1. Summary of Potential Environmental Impacts

Attribute/Measure	No Action Alternative	Deregulation in Whole
Commercial Canola Production	Glyphosate-tolerant canola already planted and traded for over a decade	Same as no action; glyphosate-tolerant canola will continue to be traded; glyphosate residue levels will fall within existing tolerance
Canola Seed Production	Seed production practices well established; cross pollination from *B. napus* possible	Same as no action; no change anticipated in seed production practices; *B. napus* cross pollination possible
Specialty Canola Production (*i.e.*, organic, modified oil, etc.)	Seed production practices in place; cross pollination from *B. napus* possible	Same as no action; no changes anticipated in specialty canola production; cross pollination from *B. napus* possible
Other *Brassica* Crop and Seed Production Including *Brassica* Vegetables	Seed and crop production practices in place; cross pollination from *B. napus* unlikely	Same as no action; no changes anticipated in *Brassica* crop and seed production including vegetables ; cross pollination from *B. napus* unlikely
Agricultural Practices	Growers will continue to use glyphosate-tolerant and other herbicide-tolerant canola	Same as no action for cropping practices, disease and insect management and volunteer management; growers may use a higher rate of glyphosate for weed management (comparable to rates used on maize, cotton, soybean) and a wider window of application
Cross pollination and ruderal canola populations	Cross pollination occurs in canola fields planted in close proximity; ruderal populations are short-lived unless the seed bank is replenished	Same as no action: no change in cross pollination from *B. napus* or establishment of populations of ruderal canola
Human Health	Canola oil will continue to be widely consumed for its health benefits; exposure to glyphosate from glyphosate-tolerant canola will continue	MON 88302 oil composition comparable to conventional control; exposure to glyphosate increases, (but comparable to current maize, cotton, soybean levels)

Table I-1. Summary of Potential Environmental Impacts (continued)

Attribute/Measure	No Action Alternative	Deregulation in Whole
Plant and Animal Communities Including Threatened and Endangered Species (TES)	Exposure of communities and TES to glyphosate will continue at a level U.S. EPA has determined is acceptable in terms of human health and environmental impact	Exposure to glyphosate in canola may increases, (but comparable to current maize, cotton, soybean levels) which U.S. EPA has determined are acceptable in terms of human health and environmental impact
Soil Microbes	Soil microbes will continue to be exposed to glyphosate	Exposure to glyphosate in canola may increases, (but comparable to current maize, cotton, soybean levels); no change in soil microbial populations anticipated
Land Use	Glyphosate-tolerant canola (RT73) will continue to be grown on land devoted to crop production; acreage is driven by demand	Same as no action; growers may replace RT73 with MON 88302 or other deregulated events; overall canola acreage not expected to change
Climate (air, water)	Growers will continue to use glyphosate-tolerant and other herbicide-tolerant canola; glyphosate will continue to be used with glyphosate-tolerant canola	May increase glyphosate use (but comparable to rates used on maize, cotton, soybean)
Non-crop and Non-agricultural Areas	Growers will continue to use glyphosate-tolerant and other herbicide-tolerant canola; areas will continue to be exposed to glyphosate drift; non-persistent populations of feral canola will continue to be established	May increase exposure to glyphosate (but comparable to rates used on maize, cotton, soybean), which U.S. EPA has determined are acceptable in terms of human health and environmental impact; non-persistent feral populations of canola will be established
Economic	Growers continue to use glyphosate-tolerant canola and derive economic benefit due to improved weed control	Same as no action; growers will use MON 88302 and derive similar economic benefits from improved weed control

Table I-1. Summary of Potential Environmental Impacts (continued)

Attribute/Measure	No Action Alternative	Deregulation in Whole
Cumulative Impacts	Glyphosate-tolerant canola and glyphosate will continue to be used; stacked glyphosate and glufosinate-tolerant canola varieties may be developed	Same as no action with t he exception that more glyphosate may be used (but comparable to what is used on maize, cotton and soybean), on the 1-1.5M acres in the U.S. currently devoted to canola production

I.2. Background and Rationale

Monsanto Company has developed a second-generation glyphosate-tolerant canola product, MON 88302, designed to provide growers with improved weed control through greater flexibility and tolerance to higher rates of glyphosate herbicide application. MON 88302 produces the same 5-enolpyruvylshikimate-3-phosphate synthase (CP4 EPSPS) protein that is produced in commercial Roundup Ready crop products, via the incorporation of a *cp4 epsps* coding sequence. The CP4 EPSPS protein confers tolerance to the herbicide glyphosate, the active ingredient in the family of Roundup agricultural herbicides.

I.3. Purpose and Need for Action

I.3.1. Regulatory Authority

"Protecting American agriculture" is the basic charge of the U.S. Department of Agriculture's (USDA) Animal and Plant Health Inspection Service (APHIS). APHIS provides leadership in ensuring the health and care of plants and animals. The agency improves agricultural productivity and competitiveness, and contributes to the national economy and the public health. USDA asserts that all methods of agricultural production (conventional, organic, or the use of genetically engineered varieties) can provide benefits to the environment, consumers, and farm income.

In 1986, the Federal Government's Office of Science and Technology Policy (OSTP) published a policy document known as the Coordinated Framework for the Regulation of Biotechnology. This document specifies three Federal agencies that are responsible for regulating agricultural biotechnology in the U.S.: USDA APHIS, the U.S. FDA, and U.S. EPA. Products are regulated according to their intended use and some products are regulated by more than one agency. The USDA, U.S. EPA, and U.S. FDA enforce agency-specific regulations to products of biotechnology that are based on the specific nature of each biotechnology-derived organism. Together, these agencies ensure that the products of modern biotechnology are safe to grow, safe to eat, and safe for the environment. APHIS regulates biotechnology-derived organisms under the Plant Protection Act of 2000 (PPA).[3] U.S. FDA regulates biotechnology-derived food products under the authority of the Federal Food, Drug, and Cosmetic Act (FFDCA).[4] The U.S. FDA policy statement concerning regulation of products derived from new plant varieties, including those genetically engineered, was published in the Federal Register on May 29, 1992[5]. Under this policy, U.S. FDA uses a consultation process to ensure that human food and animal feed safety, nutrition and other regulatory issues are resolved prior to commercial distribution of foods and feeds derived from new plant varieties. The U.S. EPA regulates plant-incorporated protectants under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and certain biological control organisms under

[3] 7 USC § 7701 et seq.
[4] 21 USC § 301 et seq.
[5] 57 FR 22984- 23005

the Toxic Substances Control Act (TSCA).[6] Together, these agencies ensure that the products of modern biotechnology are safe to grow, safe to eat, and safe for the environment.

In addition to its authority with respect to plant-incorporated protectants, the U.S. EPA also regulates the use of herbicides under FIFRA, and establishes tolerances for herbicide residues on food under the FFDCA. In order to be registered as a pesticide under FIFRA, it must be demonstrated that when used with common practices, a pesticide will not cause unreasonable adverse effects in the environment. Under the FFDCA, pesticides added to (or contained in) raw agricultural commodities generally are considered to be unsafe unless a tolerance or exemption from the requirement of a tolerance has been established. Residue tolerances for pesticides are established by U.S. EPA under the FFDCA; the U.S. FDA enforces the tolerances set by the U.S. EPA.

I.3.2. Regulated Organisms

The APHIS Biotechnology Regulatory Service's (BRS) mission is to protect America's agriculture and environment using a science-based regulatory framework that allows for the safe development and use of biotechnology-derived organisms. APHIS regulations[7], which were promulgated pursuant to authority granted by the PPA, regulate the introduction (importation, interstate movement, or release into the environment) of certain biotechnology-derived organisms and products. A biotechnology-derived organism is considered 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 the regulation (7 CFR § 340.2) and is also considered a plant pest. A biotechnology-derived organism may also be regulated under Part 340 when APHIS has reason to believe that the biotechnology-derived organism may be a plant pest or APHIS does not have sufficient information to determine if the biotechnology-derived organism is unlikely to pose a plant pest risk (7 CFR § 340.1).

A person may petition the agency under 7 CFR 340.6, "Petition for Determination of Nonregulated Status" to evaluate submitted data and determine that a particular regulated article is unlikely to pose a plant pest risk, and, therefore, should no longer be regulated. The petitioner is required to provide information under § 340.6(c)(4) related to plant pest risk that the agency may use to determine whether the regulated article is unlikely to present a greater plant pest risk than the unmodified organism. A biotechnology-derived organism is no longer subject to the regulatory requirements of 7 CFR part 340 when APHIS determines that it is unlikely to pose a plant pest risk.

I.3.3. Petition for Determination of Nonregulated Status: Monsanto 88302

In July 2011, Monsanto Company (Monsanto) has submitted a petition to APHIS seeking a determination of nonregulated status for MON 88302 (Petition #11-CA-233U). As detailed in the petition, MON 88302 produces the same 5-enolpyruvylshikimate-3-

[6] 7 USC § 136a et seq.; 15 USC § 2601 et seq.
[7] 7 CFR part 340

phosphate synthase (CP4 EPSPS) protein that is produced in other Monsanto commercial glyphosate-tolerant crop products, including Monsanto's current glyphosate-tolerant canola, RT73. The CP4 EPSPS protein confers tolerance to the herbicide glyphosate. MON 88302 allows for a wider window of glyphosate application than RT73 and higher rates of use. The extended window and higher rates of glyphosate application with MON 88302 result from an increased level of glyphosate tolerance in the male reproductive tissues of MON 88302, which is achieved through use of improved promoter sequences that regulate expression of the CP4 EPSPS coding sequence.

I.3.4. APHIS Action

Under the authority of 7 CFR part 340, APHIS has the responsibility for the safe development and use of biotechnology-derived organisms under the provisions of the PPA. APHIS must respond to petitioners that request a determination of the regulated status of biotechnology-derived organisms, including genetically engineered crop plants such as MON 88302. If a petition for nonregulated status is submitted, APHIS must make a determination if the genetically engineered organism is not likely to pose a plant pest risk.

MON 88302 has been field tested in the U.S. since 2005 under APHIS authority and oversight. Regulatory notifications of these field trials acknowledged by APHIS are listed in the petition in Appendix A. These field tests allow for evaluation in agricultural settings under confinement measures designed to minimize the likelihood of the test crop's persistence in the environment after completion of the field trial. Under confined field trial conditions, data are gathered on multiple parameters and used by the developer to evaluate agronomic characteristics and product performance. In summary, the phenotypic, agronomic, and environmental interaction data were evaluated to characterize MON 88302, and to assess whether the introduction of the trait in MON 88302 alters the plant pest potential compared to conventional canola. The evaluation, using a weight of evidence approach, considered the reproducibility, magnitude, and direction of detected differences (trends) between MON 88302 and the conventional control, and comparison to the range of the commercial reference varieties. Results from the phenotypic, agronomic, and environmental interactions assessment indicated that MON 88302 does not possess weedy characteristics, increased susceptibility or tolerance to specific abiotic stress, diseases, or arthropods, or characteristics that would confer a plant pest risk or a significant environmental impact compared to conventional canola. These data are also valuable to APHIS for assessing the potential for a biotechnology-derived plant to pose a plant pest risk.

As a Federal agency subject to compliance with the National Environmental Policy Act (NEPA),[8] APHIS must consider the potential environmental effects of its actions/decisions and reasonable alternatives to those actions, consistent with NEPA regulations[9] and the USDA and APHIS NEPA implementing regulations and

[8] 42 USC §4321 *et seq.*
[9] 40 CFR Parts 1500-1508

procedures.[10] This environmental report (ER) has been prepared to support an anticipated environmental assessment to be prepared by APHIS.

I.3.5. Other Regulatory Submissions

In accordance with the U.S. FDA's consultation policy (discussed in Petition Section I.C.1.), Monsanto submitted a food and feed safety assessment and nutritional assessment summary to U.S. FDA for MON 88302 in March 2011. In the first quarter of 2011, Monsanto also submitted a request for a joint review by the U.S. EPA and Health Canada's Pest Management Regulatory Agency (PMRA) of an amended label for use of glyphosate on MON 88302 glyphosate-tolerant canola, which incorporates higher application rates and later timing of the application. The glyphosate residue levels for MON 88302 fall within existing tolerances.[11]

As indicated herein, although certain issues related to the usage of glyphosate in conjunction with biotechnology-derived canola such as weed resistance and potential impacts of glyphosate on animals or plants are addressed by the U.S. EPA based on its authority under FIFRA, this ER also discusses those issues.

I.4. Affected Environment

I.4.1. Commercial Canola Production and Use

While canola oil can be derived from any one of three species: *B. napus, B. rapa,* and *B. juncea* (U.S. FDA, 1988; U.S. FDA, 2000; OGTR, 2008), most canola oil is derived from *Brassica napus*. Oilseed rape is a member of the mustard (Brassicaceae) family, and has been cultivated by ancient civilizations in Asia and the Mediterranean primarily for its use as oil in lamps (Colton and Sykes, 1992). Later *B. napus* oil was used as an industrial lubricant, and today there is still demand for high erucic oil in a variety of industrial applications.

Until recently, the presence of the naturally occurring toxicants, erucic acid in the oil fraction and glucosinolates in the meal has made oilseed rape oil and meal derived from *B. napus* unattractive for human consumption and as an animal feed, respectively, particularly in western countries. High erucic acid oilseed rape oil (as much as 50% of total fatty acids) has been shown to have cardiopathic potential resulting in a weakening of the heart muscle in experimental animals (Bozcali, et al., 2009; Chien, et al., 1983) and high levels of glucosinolates made oilseed rape meal unsuitable for use in animal nutrition because of anti-nutritional, goitrogenic (suppresses thyroid function), reproductive, and palatability problems (Fenwick, et al., 1989). However, in the 1960s intensive breeding programs resulted in the development and introduction of low erucic acid or canola (Canadian oil, low acid) varieties of oilseed rape (OECD, 2001; OGTR, 2008). At approximately the same time low erucic acid varieties of *B. rapa* were introduced (OECD, 2001). Slightly later, in the 1980s, low erucic acid varieties of

[10] 7 CFR § 1b and 7 CFR part 372
[11] 40 CFR § 180.364

B. juncea were developed (CCC, 1999). However, *B. napus* varieties are the most commonly grown canola oil-producing varieties in the U.S. (Boyles, et al., 2009).

In North America canola is grown primarily in prairie areas that have high quality soil, but where shorter, drier growing seasons make maize and soybean production less attractive (Figure I-1). As shown in Figure I-1, the major canola production region is primarily in Canada and extends into North Dakota.

I.4.1.1. Canola Types

There are generally three types of canola crops that can be grown in the U.S. and the grower must decide which of these types is best suited for his area and cropping system (Brown, et al., 2008). Winter canola is planted in the fall, overwinters, requires vernalization (winter-chilling) to produce flowers, and is harvested the following summer. Winter canola is generally produced in the Pacific Northwest, southern Great Plains and Midwest regions of the U.S. There is a second type of winter canola that is planted in the fall and overwinters, but does not require vernalization to produce flowers. This winter type is produced in the southeast region of the U.S. The third type is spring canola which is planted in the spring and is harvested in late summer of that same year. Spring canola is grown primarily in the northern Great Plains states including North Dakota, South Dakota, Minnesota, Montana, Idaho, and also in Washington. Winter and spring canola varieties may require different agronomic practices and can be affected by different insect pests and diseases.

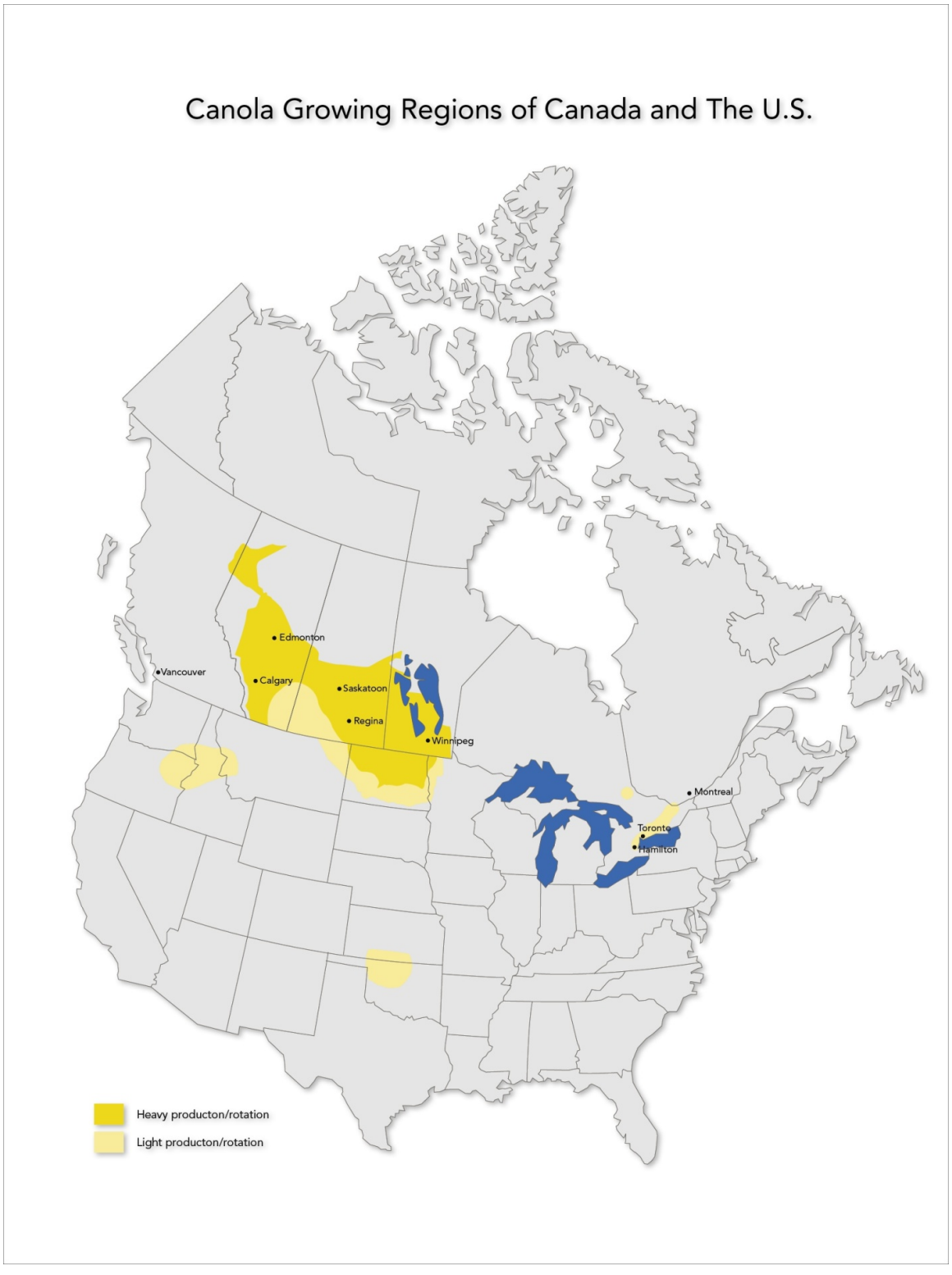

Figure I-1. Canola Growing Regions of Canada and the U.S.
Source: Canola Council of Canada http://www.canola-council.org/gallery/726/canola_growing_region_map.aspx

In a few areas, either crop may be grown. For example, Washington State University Extension staff reported in 2007 that 5,000 acres of irrigated winter and spring canola was grown in Washington, primarily in the Columbia Basin (Hang, et al., 2009). Winter and spring canola varieties require slightly different agronomic practices and can be affected by different insect pests and diseases (Brown et al., 2008). Winter canola has a higher yield potential than spring canola (Boyles et al., 2009) but can only be grown in areas with relatively mild winters.

I.4.1.2. Herbicide Tolerance

The great majority of the canola grown in North America is herbicide-tolerant. In 2008 an estimated 95% of the canola grown in North Dakota, where approximately 88% of U.S. canola is grown, and 86% of canola grown in Canada was herbicide-tolerant (USDA-NASS, 2011c; Zollinger, et al., 2009; CCC, 2010b). An additional 2% of the North Dakota crop in 2008 was tolerant to the herbicide imazamox. The imazamox tolerance was developed through conventional breeding techniques.

In North Dakota in 2008, 56% of the canola planted was glyphosate-tolerant and 39% was glufosinate-tolerant (Zollinger et al., 2009). Glufosinate-tolerant canola was commercially introduced in the U.S. in 1998 and glyphosate-tolerant canola was commercially introduced in the U.S. in 1999.

I.4.1.3. Uses

Canola oil is currently the world's third largest source of vegetable oil after palm oil and soybean oil (ASA, 2010; USDA-ERS, 2010b). Canola oil appeals to health conscious consumers because it contains a low level (7%), of saturated fatty acids which have been shown to increase blood cholesterol levels; a high level (approximately 60%) of the monounsaturated fatty acid, oleic acid, which has been shown to reduce serum cholesterol levels; a moderate level (approximately 20%) of linoleic acid, and an appreciable amount (approximately 10%) of alpha-linolenic acid, relative to other oils, (CCC, 2010a) that are essential to human health and must be supplied in the diet. Canola seed is also processed into canola meal which is used as high protein animal feed. Canola meal is the second largest protein meal source produced in the world. However, it is relatively small compared to soybean meal. Global production of canola meal was 30.8 million metric tons in 2008/2009 compared to 151.6 million metric tons for soybean meal (USDA-ERS, 2010b).

I.4.1.4. U.S. Production and Demand

In 2009-2010 the European Union, China and Canada were the largest producers of canola with 36%, 23%, and 20% of world share, respectively (USDA-FAS, 2011c). The U.S. produced 1.9% (USDA-NASS, 2011a) of the world's 60.6 million metric tons of canola in the 2010 growing season (USDA-FAS, 2011c), and imported 0.7 million metric tons from Canada (Statistics Canada, 2011).[12] Total global U.S. imports of canola oil

[12] The USDA FAS reports do not distinguish canola but report all rapeseed.

continue to increase steadily from 0.5 million metric tons in 2000 (USDA-FAS, 2011a) to 1.2 million metric tons in 2010 (USDA-FAS, 2011b).

In 2010 canola was planted on approximately 1.45M acres in the United States producing 1.1 million metric tons of canola, with a value of approximately $487 million (USDA-NASS, 2011a; USDA-NASS, 2011b). In a 2010 dollar-value comparison with other major crops, canola represents 0.7% of the value of the maize crop, 1.3% of the value of the soybean crop and 3.7% of the value of the wheat crop (USDA-NASS, 2011b). Figure I-2 shows U.S. canola production and demand from 1987 to 2009. Although canola production in the U.S. has increased dramatically since the 1980s, it has always been short of demand. The U.S. shortfall is made up with imports primarily from Canada.

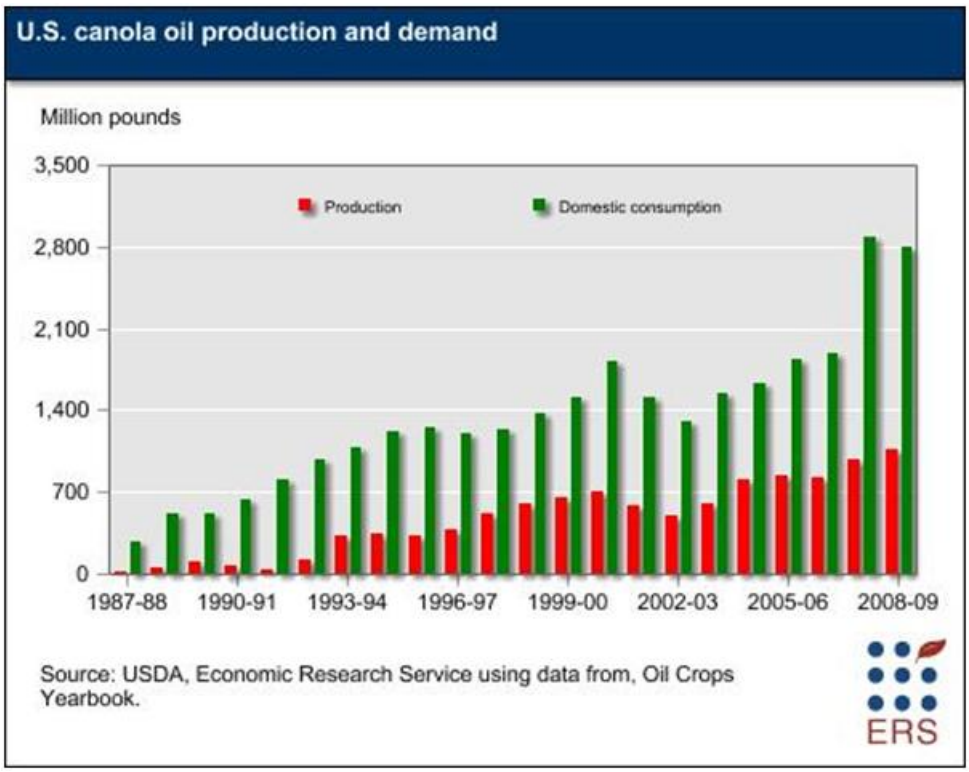

Figure I-2. U.S. Canola Oil Production and Demand
Source: USDA-ERS, 2010b.

Figure I-3 shows acres of U.S. canola planted steadily rose through the 1990s until they peaked between 2000 and 2002, and varied throughout the 2000s. The planted acres in 2010 (1.45M) were also near the 2000 peak, however, production (metric tons) was substantially higher, reflecting a trend of generally increasing yields since that time (USDA-NASS, 2011c).

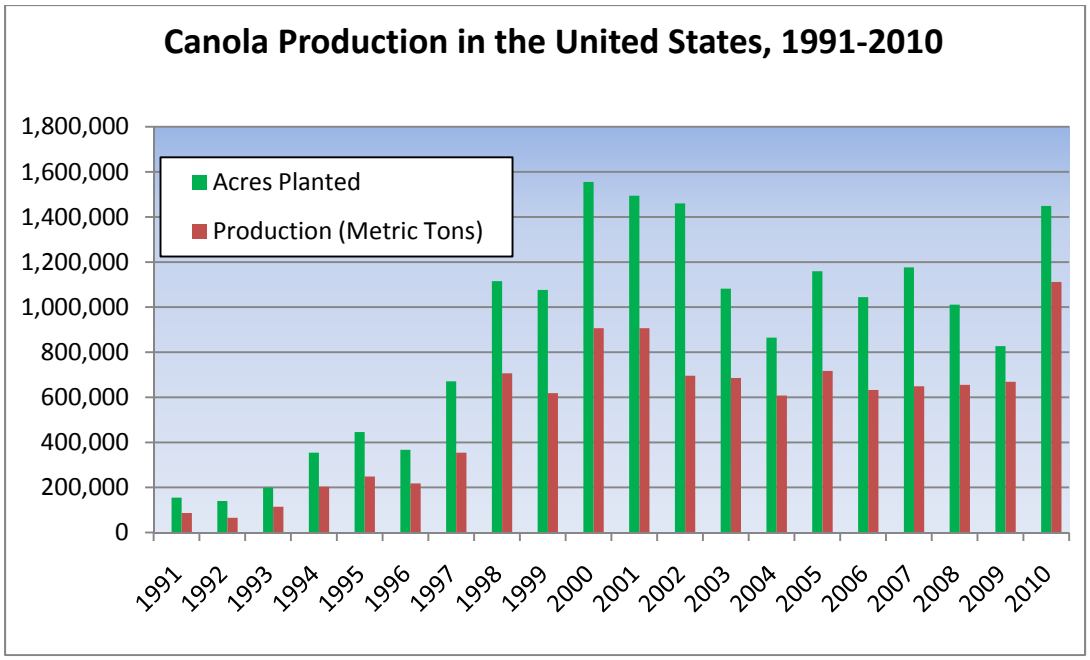

Figure I-3. U.S. Canola Harvested Seed Production 1991-2010
Source: USDA NASS 2011c.

Figure I-4 shows U.S. Canola production by state. As shown, the bulk of U.S. canola is produced in North Dakota and Minnesota. U.S. canola production is summarized in Petition Tables VIII-1 and VIII-2.

The 2007 Census of Agriculture reports canola harvested acreage in 22 states in addition to those specified in Figure I-4. In twelve of these states, canola production occurred on 3 or fewer farms and acreage was not reported (Alaska, Arkansas, Illinois, Indiana, Iowa, New York, North Carolina, South Dakota, Utah, Vermont, Virginia and Wyoming). Harvested acres were reported for the following states (harvested acres in parentheses): Washington (10,449), Kansas (3,362), Wisconsin (1,996), Colorado (1,757), Maine (1,364), Texas (486), Michigan (152), Pennsylvania (108), Nebraska (95), and California (16) (USDA-NASS, 2009).

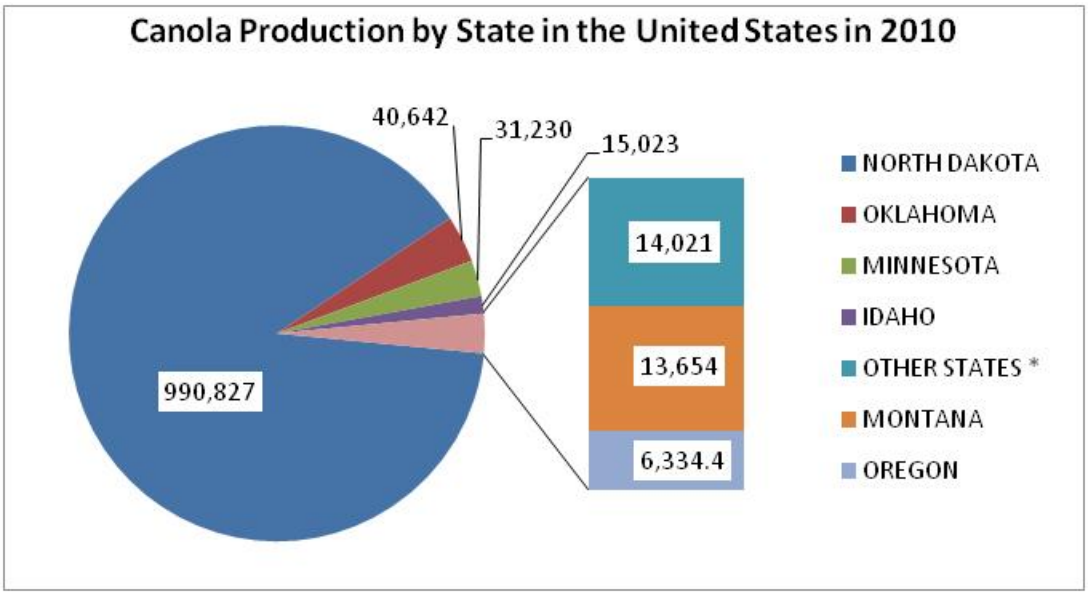

Figure I-4. U.S. 2010 Canola Harvested Seed Production by State (metric tons)
Source: USDA-NASS, 2011c. *See text for discussion of other states.

I.4.2. Seed Production for Crop Production

In addition to the need for fertile agricultural land, favorable conditions for canola seed production include dry conditions, access to irrigation, appropriate temperatures (measured as heat units that are determined by the maximum and minimum daily temperature through the growing season), and adequate distance from commercial canola production areas (Monsanto Company, 2009). Based on these constraints, most of the canola seed produced for sale for crop production for the North American market is produced in the summer in a small geographic area in southern Alberta, Canada and the northwestern U.S., with the bulk of the production in the area around Lethbridge, Alberta (Monsanto Company, 2009; Bewley 2008). Among the areas of U.S. summer seed production are the Columbia Basin in eastern Washington, the Grand Ronde Valley in Union County in northeastern Oregon, the Magic Valley along the Snake River Plain in south-central Idaho, the Idaho Falls area in far eastern Idaho near the Wyoming border, the Park River area of northeastern North Dakota (and possibly northwestern Minnesota) in the Red River Valley, and the San Luis Valley in south central Colorado (Bateman, Monsanto Company, Personal Communication, 2011; Bewley 2008).

To meet the demand for seed and to minimize production risks, most seed companies have off-season seed production locations in the southwestern U.S. (Imperial Valley, California and Yuma Valley, Arizona) and/or Chile, with the Central Valley of Chile the main off-season location (Monsanto Company, 2009, Bateman, Monsanto Company, Personal Communication, 2011). Canola seed production is discussed in more detail in Petition Section VIII.B.2.

I.4.2.1. Canola Seed Needed for Crop Production

Based on an average canola seeding rate of five pounds per acre, approximately 7.5 million pounds (3,400 metric tons) of canola seed is required to plant the approximately 1.5 million acre U.S. crop (Monsanto Company, 2009). With allowances for seed losses due to weather, poor yields, quality issues, distribution excess, seed returns and replants, approximately 5,000 acres of commercial seed production are needed to supply sufficient seed to plant the entire U.S. canola acreage.

I.4.2.2. Certified Seed Production

Many growers choose to use certified seed when planting their fields. Certified seed is seed of a known variety produced under strict state and federal seed certification standards to maintain varietal purity. Seed lots must also meet specified standards for other crop seed, inert matter, weed seeds, and germination. All certified seed must pass field inspection and be sampled and pass laboratory testing before it can be labeled and sold as certified seed. In the U.S. the state seed certifying agencies are often associated with land grant universities. These various state agencies along with certifying agencies from other countries are members of the Association of Official Seed Certifying Agencies (AOSCA). The AOSCA coordinates the efforts of seed certification agencies, which, through the certification process, establish standards for genetic purity and seed quality (AOSCA, 2011).

AOSCA identifies three seed classes: 1) breeder; 2) foundation; and 3) certified. Breeder seed is seed directly controlled by the originating or sponsoring plant breeding organization or firm responsible for the maintenance of that variety. Foundation seed is first-generation seed increased from breeder seed and is handled to maintain specific varietal purity and identity. Certified seed is the progeny of breeder or foundation seed, and is the class recommended for commercial canola production. AOSCA standards for certified canola seed require all seed fields be inspected in the early flowering stage to ensure the fields are isolated from other canola crop fields by at least 660 feet for the production of open-pollinated varieties and 2,640 feet for production of hybrid varietal seed (AOSCA, 2009). Inspections ensure that the number of off-type plants and plants of other varieties do not exceed 1.5 plants per 10,000 canola plants. Plants of other *Brassica* crop species must not exceed 1 plant per 10,000 canola plants. The percentage of hybrid seed shall not be less than 75%.

State seed certification standards and isolation distances vary slightly from state to state and can be more restrictive than the seed standards of AOSCA (Table I-2). In states where canola seed is produced, foundation seed fields must be isolated from canola and other cross pollinated varieties by 1,320 feet to 2 miles, while certified fields must be isolated by 660 feet to 2 miles. In order to cultivate foundation canola seed, no canola or oilseed rape must have been grown on the land in the previous three to five years depending on the variety and state. This field history restriction is utilized to limit the spontaneous germination and sprouting of seeds left in the seedbank from previous canola or oilseed rape cultivation. All volunteer plants and noxious weeds must be eradicated and definite boundaries to the field set before field use.

Note that Table I-2 does not include all standards for all states listed; different states have requirements not shown in the table.

The first stage or pre-foundation seed stage occurs in isolation tents for complete pollination control. Individual inbred plants are grown and tested to ensure the plants meet the desired genetic characteristics. The next stage or foundation seed stage occurs in highly isolated open fields. During this stage seed lines are further tested to ensure the desired genetic characteristics and purity are maintained under field conditions.

I.4.2.3. Production of Open-Pollinated and Hybrid Seed

Producing hybrid canola requires using a pollination control system to prevent unwanted self-pollination. Three primary pollination control methods used in North America: 1) the biotechnology derived male sterility system; 2) the NPZ MSL (Lembke) system; and 3) the INRA-*ogura* cytoplasmic male sterility (CMS) system (Stiewe, et al., 2010). The biotechnology-derived system uses tapetal cell-specific male sterility and fertility restorer genes to facilitate hybrid seed production (Mariani, et al., 1992). The MSL-system (Male Sterility Lembke) is a private system owned by NPZ/Lembke based on a spontaneous mutant selected in the NPZ nursery (Frauen and Paulmann, 1999). The INRA-*ogura* system uses a CMS and restorer gene transferred from *Raphanus (*Buzza, 1995). The CMS system is composed of three lines, usually referred to as the A line (male sterile or female parent line), B line (maintainer line) and R line (restorer or male parent line). The A and B lines have similar genotypes, with the exception of the CMS gene, that confers male sterility to the A line. The B line has a cytoplasm which allows normal pollen production facilitating the breeding process (Buzza, 1995). The first step in the hybrid seed production process involves foundation seed crossing block where female parent Line A is pollinated by the genetically identical Line B to produce sufficient quantities of Line A seed. The second step involves pollination of Line A with restorer Line R to produce fully fertile F1 hybrid seed (Thomas, 2003).

Table I-2. Certified Canola Seed Standards

Category	Characteristic	Colorado[a] (Hybrid/Non-hybrid Varieties)	Idaho[b]	Minnesota[c] (Hybrid/Non-hybrid Varieties)	North Dakota[d]	Oregon[e]
Field Isolation Distances	Fields of any Variety of the Same Kind (feet)	2,640 / 660	660	2,640 / 660	660	2 miles
	Fields of any Variety from Another Kind (feet)	NA / 660	330	NA / NA	660	NA
Field Standards	Years Field Has Not Produced Oilseed rape, Mustard and/or Canola	5 / 3	3	5 / 3	3	5
	Ratio of Plants - other varieties	1.5:10,000 / 1:500	NA	1.5:10,000 / 1:500	1:500	1.5:10,000
	Plants of other *Brassica* & Inseparable Other Crops	1:10,000 / 1:10,000	NA	1:10,000 / 1:10,000	1:500	1:10,000
Seed Standards	Pure Seed (Min)	99% / 99%	99%	99% / 99%	99%	99%
	Inert Matter (Max)	1% / 1%	1%	1% / 1%	1%	1%
	Other Crop Seeds (Max)	0.25% / 0.25%	40/50 kg	0.25% / 0.25%	0.25%	0.01%
	Weed Seeds (Max)	33/kg / 33/kg	400/50 kg	0.10% / 0.10%	33/kg	0.25%
	Objectionable Weed Seeds (Max) [2]	11/kg / 11/kg	40/50 kg	31/kg / 31/kg	11/kg	2/sample
	Sclerotia bodies (Max)	15/kg / 15/kg	None	15/kg / 15/kg	15/kg	NA
	Germination Rate (Min)	85% / 85%	85%	85% / 85%	85%	85%

[a] Colorado Seed Growers Association (CSGA, 2010).
[b] Idaho Crop Improvement Association (ICIA, 2006).
[c] Minnesota Crop Improvement Association (MCIA, 2010).
[d] North Dakota State Seed Department (NDLB, 2008).
[e] Oregon Seed Certification Service (OSU, 2008).

I.4.2.4. Canola Seed Manufacturing

Canola seed is produced by several companies that produce and sell seed, such as Monsanto Company, Pioneer Hi-Bred International, Bayer CropScience, Croplan Genetics, and Dow AgroSciences. Seed companies contract with growers to produce the specified amount of canola seed. Seed companies have processing facilities and/or a network of contractors to clean, condition, and bag the harvested canola seed as well as monitor and inspect all facility processes.

For most of the major seed companies, the entire seed production process is International Organization for Standardization (ISO) certified and; therefore, includes internal and external audits (ISO, 2009). The ISO standards represent an international consensus on good management practices with the aim of ensuring that the organization can consistently deliver excellent product or services.

While field operations and management practices for producing canola seed are similar to those used for canola crop production, additional measures specific to seed production are sometimes needed and are described briefly below.

A seed production field should not have been planted with canola the preceding five years in order to avoid volunteer canola plants and to ensure genetic purity. All seed stock is treated with fungicides and insecticides to protect seedlings from various seedling diseases and flea beetle. Very early planting into cold soil conditions should be avoided because this can result in poor emergence and uneven stands. Every effort must be made to eliminate weeds in a seed field through the use of herbicides, cultivation and hand weeding to prevent weed seed in the harvested canola seed. Roguing is performed on all seed production fields to remove volunteer canola and off-type plants prior to or during flowering. Fields are scouted frequently for insect pests and insecticides are applied when insect pest infestations reach economic threshold levels. Foliar-applied fungicides should be considered when disease infestations are predicted in the area. Honey bee hives are placed in the seed fields to encourage pollination of the female parent line with pollen of the male parent line. Swathing (cutting the crop and placing it in rows directly on the cut stubble to facilitate drying rate, ensure even ripening and reduce the possibility of seed losses from wind and hail) and combining at harvesting should occur at optimum times to avoid seed shattering and damage to the seed. With large acreage of canola, swathing should start when seed color change is approximately 20% to 25% and the majority of the crop can be cut at or near the optimum seed color. Swathing can begin as early as 15% seed color change (NDSU, 2005). Combining should begin when the seed moisture drops below 10% and no green pods are visible (Boyles et al., 2009). Harvesting equipment must be adjusted to minimize or avoid seed damage and must be cleaned before entering the seed fields to minimize genetic contamination. Certain handling equipment, such as auger elevators, should be avoided because they can increase seed damage.

Field inspections are vital to ensure canola seed meets seed certification requirements, ISO certification standards, regulatory standards and trait licensing agreement standards. Field inspections are conducted on seed production fields throughout the growing season

to evaluate variety purity, ensure canola plants are developing properly and fields are maintained free of weeds, insects and diseases.

Production plant personnel make every effort to avoid mechanical damage to the harvested seed during the screening, cleaning and bagging process. Specific methods are used to assure the genetic purity and identity of the seed is maintained throughout the handling and storage operation. Bin inspections and sample collections are conducted at storage locations at the plant to examine the physical characteristics of the canola seed and to ensure proper bin cleanout. Seed is inspected for appearance, disease, discoloration, seed coat, mechanical damage, inert matter and weed seed. Germination, hybridity and impurity are tested and quantified on all seed lots to verify acceptable levels and meet labeling requirements.

I.4.2.5. Variety Trials

Many varieties are available for each canola type and growers rely on variety performance trials conducted by universities and private companies across a number of locations and years to help assist them in making variety selections. The North Dakota State University (NDSU) Extension Office, for example, has conducted variety trials for many years, and publishes the results each year. The 2010 trials included 58 varieties: 49 glyphosate-tolerant varieties (representing ten different seed companies), five glufosinate-tolerant varieties (one seed company) and four imidazolinone (imazamox)-tolerant varieties (three seed companies). Researchers obtain and report the following data by variety and location, for growers to use in variety selection: first flower, flower duration, maturity, seed weight, seed yield, 3-year average for seed yield (if applicable), plant height, and lodging (Kandel, 2010).

I.4.3. Specialty Canola Production

Specialty canola products, which include high erucic acid, high oleic and high oleic, low linoleic as well as organic canola require identity preservation throughout the process from seed to end product, including planting, harvesting, transporting, processing, and marketing in order to preserve their value. While standard seed production practices preserve variety identity through planting, segregation in the remainder of the production process (including the need for special cleaning of harvesting and transportation equipment and custom crushes) adds incremental costs that, for the production to be worthwhile, must be recovered in a price premium for the end product (Phillips and Smyth, 2003).

In some cases, and particularly for organic production, specialty production is required which utilizes oil processing facilities other than the very large, regional facilities where commodity canola is processed. Reproducing the complex multi-step process used in the larger facilities would be cost-prohibitive on a small scale. Small-scale specialty canola, including organic, is generally processed using a screw-type mechanical press (expeller). Expellers are available in a range of sizes suitable for commercial production to backyard use.

I.4.3.1. Organic Canola Farming

Organic farming operations as described by the National Organic Program (NOP), which is administered by USDA's Agricultural Marketing Service (AMS), requires organic production operations to have distinct, defined boundaries and buffer zones to prevent unintended contact with prohibited substances or products of excluded methods from adjoining land that is not under an organic production management plan.[13] Organic production operations must also develop and maintain an organic production system plan approved by an accredited certifying agent. This plan enables the production operation to achieve and document compliance with the National Organic Standards, including the prohibition on the use of excluded methods.[14] Excluded methods include a variety of methods used to genetically engineer organisms or influence their growth and development by means that are not possible under natural conditions or processes. The use of biotechnology such as that used to produce MON 88302 is an excluded method under the National Organic Program.[15]

Organic certification involves oversight by an accredited certifying agent of the materials and practices used to produce or handle an organic agricultural product. This oversight includes an annual review of the certified operation's organic system plan and on-site inspections of the certified operation and its records. Although the National Organic Standards prohibit the use of excluded methods, they do not require testing of inputs or products for the presence of excluded methods. The presence of a detectable residue of a product of excluded methods alone does not necessarily constitute a violation of the National Organic Standards. The unintentional presence of the products of excluded methods will not affect the status of an organic product or operation when the operation has not used excluded methods and has taken reasonable steps to avoid contact with the products of excluded methods as detailed in an approved organic system plan. Organic certification indicates that organic production and handling processes have been followed, not that the product itself is "free" from any particular substance. As USDA AMS has recently re-iterated in a policy memorandum, organic certification is process based. The NOP regulations do not allow the use of excluded methods such as biotechnology; however, the inadvertent presence of products of biotechnology "does not constitute a use because there was no intent on the part of the certified operator to use excluded methods" (USDA-AMS Organic, 2011).

Many crop management practices are similar to those used by non-organic canola growers, except that organic growers may not use synthetic fertilizers or pesticides. Because synthetic herbicides are not allowed, organic growers use cover crops and/or tillage to control weeds (Myers, 2002).

Organic producers use production practices designed to prevent commingling of their crop with neighboring crops treated with herbicides and other pesticides (spray drift), or that may be using plant varieties produced by excluded methods (pollen movement).

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[13] 7 CFR 205.202(c)
[14] 7 CFR Part 205.
[15] 7 CFR § 205.2.

These well established practices include isolation zones, use of buffer rows surrounding the organic crop, adjusted planting dates, and varietal selection (Kuepper 2006).

I.4.3.2. U.S. Organic Canola Production

Comprehensive nationwide data on organic production in the U.S. was collected for the first time as part of USDA's 2007 Census of Agriculture.[16] The USDA reported 8 farms in the U.S. in 2008 that produced organic canola, with 2 farms in Iowa, one in Michigan, 2 in New York and 3 in Washington. A total of 184 metric tons of canola with a value of $92,752 was harvested on 232 acres in 2008 (USDA-NASS, 2008). To avoid reporting data for individual farms, the only state-specific data reported was 8.5 metric tons harvested in Washington, with a value of $3,560. Based on the 2007 Census of Agriculture, there were only two farms in Iowa and two in New York that harvested canola (USDA-NASS 2009); these are likely the four organic canola farms in those states reported in 2008. The census reported 5 farms growing canola in Michigan and 56 in Washington, the other two states with organic canola production in 2008 (USDA-NASS 2009).

There is little specific information available about organic canola production in the U.S. before the USDA 2008 organic census. USDA-ERS has data on certified organic oilseed acreage by state for 1997 and 2000 to 2008. However, only the larger organic crops (flax and sunflowers) are differentiated; canola is grouped in the "Other" category along with safflower and unclassified acreage (USDA-ERS, 2010a).[17] In 1997, the year glufosinate-tolerant canola was introduced in the U.S. and the year before the introduction of glyphosate-tolerant canola, USDA-ERS reported 12,487 acres of "Unclassified/other" organic oilseed production, of which 35% (4,411 acres) was in California and 39% (4,857 acres) was in Utah. Based on other sources, for both states, this unclassified/other acreage was probably mostly, if not entirely, safflower. California (994 acres) and Utah (3,454 acres) were the only two states with reported acreage for safflower in the 2008 organic census (USDA-NASS 2008). The State of California has data for organic oilseed production for jojoba, safflower, sesame, sunflower and "other" for 2000 to 2009 (Klonsky and Richter, 2007; Klonsky and Richter, 2011). California safflower acreage for that period ranged from zero to 4,875 acres. For the period 2000 to 2009, safflower was by far the largest California organic oilseed crop, with jojoba second. The reported acreage for the "other" category was zero for 6 of the 10 years, and except for a high of 285 acres in 2002, ranged from 4 to 97 acres in the other years. If any canola had been grown in California during the reported time period, it would have been included in the "other" category.

While organic canola may have been grown in North Dakota in the past, we found only two North Dakota growers who previously grew it or considered growing it, both of them long-term organic farmers. One grower has a 3,500-acre farm in Windsor, North Dakota, that he has operated as organic since 1980 (Leopold Center for Sustained Agriculture

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[16] The organic survey was part of the 2007 census, but was done in 2008. Data were collected by mail, with a overall response rate of 87%. The mailing list was developed primarily through data from the 2007 Census of Agriculture.
[17] USDA ERS data is obtained through USDA-accredited State and private certification groups.

2000). The grower reported that canola had been a "very good crop" in his rotation, but that he had previously made a firm decision that he would not grow any crops on his farm that "have a counterpart that has a GMO crop." Therefore, when GE canola was introduced in the U.S., he stopped growing canola (Kirschenmann, 2009). The other grower has a 640-acre farm in the Red River Valley in eastern North Dakota and grows organic wheat, clover, sunflowers, rye, barley, flax, alfalfa and crambe (an oilseed crop), which he has chosen to grown instead of canola. According to the grower, crambe is better suited for organic production than canola because it is resistant to diseases and tolerant of flea beetles (Bowman, 2002).

I.4.4. Other Specialty Canola Production

A fairly recent development in canola production is the development of high-oleic canola varieties (CCC, 2006a), which produce an oil higher in monounsaturated fat and lower in polyunsaturated fat. High-oleic canola oil is more stable than commodity canola oil, allowing for a longer shelf life and greater heat tolerance than traditional or commodity canola oil (CanolaInfo, 2007). High-oleic canola oil is sold most commonly to food companies and food service operations (CanolaInfo, 2007). In the 2010 NDSU variety trials, five of the 58 varieties tested were high-oleic (Kandel, 2010).

Canola products labeled "GMO-Free" have no detectable presence of genetically modified material, and may be specialty products, depending on how they are produced. Some products, such as oils, are labeled GMO-Free even though they may or may not have been made from genetically engineered crops. Since oil does not contain proteins no detectable levels of genetic material are present (North Dakota Organics, 2011).

I.4.5. Other *Brassica* Production

This section describes *Brassica* crops other than canola that are grown in the U.S. Because of potential concerns with canola related to cross pollination and spread of disease among related species, this section focuses on seed production for vegetables and *Brassica* crops grown for seed such as mustard.

I.4.5.1. *Brassica* Vegetable Production

The primary *Brassica* vegetable crops in the U.S. are *B. napus* (rutabaga and Siberian kale), *B. oleracea* (cabbage, broccoli, cauliflower, Brussels sprouts, collards, kale, kohlrabi), *B. juncea* (red giant mustard, sawtooth mustard, and others) and *B. rapa* crops (Chinese cabbage, pak choi, choi sum, turnip, mizuna, mibuna, tat soi and others). Also grown are radish, daikon, and arugula, which are not *Brassica* species, but are in the Brassicaceae family. All *Brassica* vegetables grown in the U.S. are harvested in the vegetative stage and are therefore not of concern for cross pollination with canola. *Brassica* species represent approximately 6.8% of the total vegetable acreage in the U.S. (USDA-NASS, 2009). The 2007 Census of Agriculture reports 322,591 acres of harvested *Brassica* vegetable crops in the U.S., with broccoli accounting for 40% of the

harvested acreage and head cabbage (cabbage) and cauliflower combined accounting for and additional 37% of the harvested acreage (USDA-NASS, 2009).[18] Broccoli is commercially grown in most states, with the majority (106,271 acres or 81%) in California. The next-highest production state in 2007 was Arizona (11,869 acres or 9% harvested), then Oregon with 1,410 acres or 1% harvested. All other states had less than 1,000 acres harvested (USDA-NASS, 2009). The distribution of cauliflower production is similar to that of broccoli, with 82% of harvested acres in California, and with Arizona and Oregon with the next highest production. California is also the largest producer of cabbage (14,099 acres harvested or 17% of the total), just ahead of New York (13,618 acres). Florida, Georgia, North Carolina, Wisconsin and Texas are also major cabbage producers. California is also the leading producer of Brussels sprouts, Chinese cabbage and kale. Georgia is the leading producer of collards and Michigan leads in turnips.

I.4.5.2. *Brassica* Vegetable Seed Production

Because of the wealth of literature and detail involved, the description of *Brassica* and related vegetable seed production is included as Attachment 1. The majority of the *Brassica* vegetable seed production occurs in three distinct areas: the Willamette Valley in Oregon; Skagit County in western Washington; and the Columbia Basin in eastern Washington (Bateman, Monsanto Company, Personal Communication, 2011). These areas have strict regulations or prohibitions on growing canola. *Brassica* vegetable seed crops are also produced in California and Arizona, and broccoli seed is grown primarily in California and Arizona. According to the 2007 Census of Agriculture, canola was grown on 16 acres in California and was not grown in Arizona.

I.4.5.3. Other *Brassica* Crops Grown for Seed

Brassica and related field crops in addition to canola include high erucic acid oilseed rape, mustard seed and biofumigant mustard seed.

To meet industry requirements, industrial grade oil from oilseed rape must contain at least 45% erucic acid. It is used primarily as a lubricant, an additive in plastic, and for birdseed (USDA-ERS, 1996). It was grown on 2,200 acres in the U.S. in 2010, with a total crop value of $975,000 (USDA-NASS, 2011b; USDA-NASS, 2011c). In 2007, USDA reported 1,060 acres harvested by 11 operators, 9 of whom were in Idaho. Oregon and Washington each had one operator. No breakdown of acreage by state was reported (USDA-NASS, 2009). Partly to prevent cross pollination between industrial oilseed rape and canola, these three states have implemented regulations on where canola can and cannot be grown (Appendix Section I.4.6).

Mustard seed was harvested on approximately 48,000 acres in 2010 (USDA-NASS, 2011c). In 2007, mustard seed was grown in 10 states with North Dakota, Montana,

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[18] Other *Brassica* crops reported include Brussels sprouts, Chinese cabbage, mustard cabbage, collards, kale, mustard greens, turnips, and turnip greens, with harvested acreages ranging from 66 acres (mustard cabbage) to 11,480 acres (Chinese cabbage). Other vegetable crops in the Brassicaceae family include daikon (624 acres), horseradish (3,692 acres), radishes (14,599 acres) and watercress (679 acres).

Washington and Idaho together accounting for 98% of the acreage (61%, 25%, 9% and 3%, respectively). In North Dakota mustard is grown in rotation with small grains, but is not recommended for close rotation with canola because of similarity of disease susceptibility (NDSU, 2007b). The most common type grown is yellow mustard (*S. alba*), which is used as condiment mustard. Only small acreages of brown and oriental mustard (*B. juncea*) are grown; these are used for oils and spices (NDSU, 2007b).

Biofumigant mustard is used as a green manure crop (crop is grown then plowed into the soil at different times in the growing season). As it decomposes it releases naturally-occurring compounds that are toxic to some weeds, nematodes and fungi (McGuire, 2003). Biofumigant mustard species are the same as those used for mustard seed crops (*S. alba* and *B. juncea*).

I.4.6. State Restrictions on *Brassica* Crops

Oregon, Washington and Idaho all have some restrictions (spatial, crop rotation, etc.) on growing canola and some other *Brassica* seed crops. No other states were found that have state-based restrictions on growing canola and other *Brassica* crops. These restrictions are described in this section. Part of the reason for these restrictions is to prevent cross pollination between canola and industrial oilseed rape. There is little industrial oilseed rape grown in these states today; however, in the 1980s and early 1990s industrial oilseed rape acreages were substantially higher (USDA-ERS, 1996). As discussed in Attachment 1 these states all have *Brassica* and related vegetable seed production. Blackleg, a fungal disease common to *Brassica* and related species, is widespread in most canola producing areas, but has not been introduced in Oregon, Washington or Idaho. Thus the regulations are also intended to help keep blackleg out of these states. Finally, given the potential for cross pollination between sexually compatible *Brassica* species, these states have also adopted regulations to limit cross pollination between canola and *Brassica* vegetable crops in vegetable seed production areas.

I.4.6.1. Oregon Restrictions

Since 1989, the Oregon Department of Agriculture has had the authority under Oregon law to establish control areas for the production of oilseed rape (including canola) if it determines control is necessary for protection of "horticultural, agricultural or forestry industries of the state from diseases, insects, animals or noxious weeds…."[19] The Department of Agriculture first implemented regulations with oilseed rape growing restrictions in 1992 for the purpose of preventing cross pollination between canola (edible oil) and industrial oilseed rape.[20] The regulations were rewritten in 2005 "to account for

[19] Oregon Revised Statues (ORS) §570.450 and §570.405.

[20] Oregon regulations 603-052-0860(8) define rapeseed as follows: "Rapeseed" means plants of the species *Brassica napus*, *Brassica rapa* and *Brassica juncea*, where seeds of high oil content are the economically valuable product. Included are the industrial seed types, with high erucic acid levels and canola with low erucic acid content used for edible oils.

conflicts between specialty seed production and canola [oilseed rape] grown for biodiesel or edible oil." The Department of Agriculture made small modifications to the regulations in 2009 and will be reviewing them again in 2012 (ODA, 2011). The fungal disease blackleg (*Leptosphaeria maculans*), which is a serious disease in the major North American canola production areas, is not present in the Pacific Northwest (Brown et al., 2008), and the regulations includes requirements for seed production (described below) to help prevent blackleg's introduction into Oregon. Long distance spread of blackleg is usually by infected seed (NDSU, 2004).

The regulations establish a "general production area" and four "protected districts."[21]

General Production Area: All areas of the state not in a protected district are in the general production area. The following restrictions apply to all oilseed rape grown within the general production area:

• All oilseed rape seed that "trades within commerce" must be certified by AOSCA and state standards, the seed must be certified to be free from blackleg and must be treated with a fungicide approved for blackleg control.

• Oilseed rape cannot be grown in the same plot more often than two years (non-consecutively) in five.

• All unbagged loads of oilseed rape transported through protected districts "must be in enclosed bins or in containers lined and covered in a manner to prevent seed loss."[22]

Protected Districts: The Willamette Valley Protected District includes the Willamette Valley. The Central Oregon Protected District includes parts of Crook, Jefferson and Deschutes counties. The Northeast Oregon Protected District includes part or all of Baker, Union and Wallowa counties. The Malheur Protected District includes a 3 mile strip of northern Malheur County adjacent to the Idaho border (ODA, 2011). Within all the protected districts except the Northeast District, oilseed rape production for seed or oil is prohibited except under special permit. Oilseed rape production for seed or oil is allowed in the Northeast District without a permit. The same restrictions applicable to the general production area apply to production in the protected districts, except that oilseed rape can be grown on the same plot of land only once every four years. The following additional restrictions apply to all protected districts:[23]

- Oilseed rape "must be isolated from other crops with which it will cross pollinate, by a distance of not less than three miles," except that in the Northeast District, the isolation distance is two miles.
- The locations of oilseed rape fields must be recorded with the Oregon State University County Extension Office, or in the Willamette Valley, with the Willamette Valley Specialty Seed Association (WVSSA), at least 10 days prior to planting.

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[21] Oregon regulations: 603-052-0850. Map is available on website. See Oregon Department of Agriculture 2011.
[22] 603-052-0880(1)
[23] 603-052-0880(2)

- Forage and cover crop oilseed rape shall not be allowed to flower.
- Any volunteer or uncontrolled oilseed rape "in and around production fields must be prevented from flowering by the producer."
- Violators may be fined or subjected to crop destruction.[24]

I.4.6.2. Washington Restrictions

Since 2007, Washington state law allows the State Department of Agriculture to establish *Brassica* seed production districts, with restrictions on production of *Brassica* seed.[25] The law is broad and allows the Department of Agriculture to make specific restrictions. The State Department of Agriculture has established two *Brassica* seed production districts. Seed Production District 1 includes all or parts of Whatcom, Skagit, Snohomish, Island and Clallam counties (in western Washington) and Seed Production District 2 includes parts of Grant and Adams counties.[26]

Within both *Brassica* seed production districts, all *Brassica* seed crops grown for seed or oil may be grown only through participation in the Washington State University Extension Center pinning process. Within both districts, seed must be transported in "covered containers from which the seed cannot leak" and volunteers must be controlled "as soon as feasible but prior to pollen production or blooming."[27]

In *Brassica* Seed Production District 1, oilseed rape grown for seed or oil is prohibited unless grown under the conditions of a *Brassica* production agreement.[28] A production agreement must be developed through the director of the State Department of Agriculture and an advisory committee and the agreement must contain "terms and conditions that are necessary and sufficient to mitigate reasonably possible risks to the economic well-being of growers within the *Brassica* seed production district from the proposed activity". The agreement is subject to appeal by any grower or processor within the *Brassica* production district.[29]

In *Brassica* Seed Production District 2, a 2-mile isolation distance is required between *Brassica* seed crops, except for certain situations where growers make specific written agreements.[30] A part of *Brassica* Seed Production District 2 (2A) has an additional restriction: *Brassica* seed crops "intended for oil or fuel production" may be planted only under the conditions of a *Brassica* production agreement.[31]

I.4.6.3. Idaho Restrictions

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[24] 603-052-0880(6)
[25] RCW 15.51.030
[26] Washington regulations 16-326-010
[27] Washington regulations 16-326-020
[28] Washington regulations 16-326-030
[29] RCW 15.51.40
[30] Washington regulations 16-326-040
[31] Washington regulations 16-326-050

Idaho has had restrictions on oilseed rape production since 2005. The Idaho regulations divide the state into seven districts. Within four of these districts, only edible oilseed rape (canola) production is allowed, within two only industrial oilseed rape production is allowed, and within one (District IV) no oilseed rape production is allowed.[32] District IV covers Treasure Valley and includes Ada, Gem, Canyon, and Payette counties and part of Owyhee County, in western Idaho. Except for District IV, exemptions are allowed for planting the restricted oilseed rape with minimum one-mile isolation and written agreement from all adjacent farmers.

Volunteer oilseed rape growing outside of cultivated fields "shall be destroyed before flowering" by "the person responsible for planting the oilseed rape."[33] Idaho regulations require that any *Brassica* seed transported in Idaho be sealed or covered to avoid spillage.[34] Idaho also has requirements similar to Oregon's to prevent the introduction of blackleg fungus into Idaho.[35]

I.4.7. Agronomic Practices for Canola

A grower's goal in canola production is to produce a crop with high yield, high oil content, low green seed content, low weed seed content, and little seed left in the field, all while managing input costs. This section discusses those practices that are within a grower's control that help achieve this goal. Agronomic practices are discussed in detail in Petition Section VIII.

I.4.7.1. Production Costs

Managing input costs is a major component to the economics of producing a profitable canola crop. The key decisions on input costs include choosing what seed or canola varieties to plant, amounts of fertilizer to apply and what herbicide program to use. The average total income for producing canola in North Dakota was $250.89 per acre for the years 2005-2009 according to statistics compiled by Farm Management Specialists (FINBIN, 2010). The total direct and overhead expenses were $179.52 and $27.44 per acre, respectively. Major direct per acre expenses were fertilizer ($43.56), land rent ($34.50), seed ($31.06) and crop chemicals ($22.69). The net return for the five-year period averaged $43.94 per acre. With an average government payment of $11.33 per acre the net return was $55.27 per acre. For more detailed information see Table VIII-3 in the Petition.

I.4.7.2. Tillage

Canola can be grown with conventional tillage, conservation tillage, or direct-seeded into small grain straw stubble in a no-tillage cropping system (Brown et al., 2008). The benefits of conservation tillage or no-till systems in canola production include reduced

[32] Idaho regulations 02.06.13 §50 and §100
[33] Idaho regulations 02.06.13 §200
[34] Idaho regulations 02.06.13 §250
[35] Idaho regulations 02.06.13 §150

soil erosion, reduced fuel and labor costs, conservation of soil moisture, improvement of soil structure, reduction of soil compaction and improvement of soil organic matter content (Brown et al., 2008).

Spring canola has provided good performance under conservation tillage while winter canola has generally performed poorly with conservation tillage systems (Brown et al., 2008). Winter canola is not recommended when planting into excessive amounts of fresh straw, or when soil temperatures are lower than average in the fall months. This can result in poor emergence. Seed yields with direct-seeded winter canola have been significantly lower than with conventional tillage systems (Brown et al., 2008). Spring canola is better suited to conservation tillage due to better soil moisture conservation and availability. Spring canola has fewer problems dealing with heavy straw residue and, residue from the previous crop has usually decomposed to some degree during the winter months prior to planting spring canola. In addition, cooler soil temperatures can be advantageous to spring canola. The Conservation Tillage Information Center (CTIC, 2008) reports that conservation tillage (no-till or mulch-till) is used on approximately 32% of the canola acres in North Dakota. Crop specialists at North Dakota State University indicate that the amount of direct-seeded or no-till canola in North Dakota varies across the state based on rainfall. In the eastern or Red River Valley area where rainfall is relatively high, only about 5% is direct-seeded into wheat straw (Kandel, NDSU, Personal Communication, 2010). The drier, central area and even drier, western area of the state are estimated to have approximately 80% and 100% of the acreage direct-seeded, respectively.

I.4.7.3. Planting

Canola is typically seeded in 6 or 7-inch rows with a grain drill or air seeder, with optimal seeding depths between one half to one inch. Canola is very susceptible to soil crusting, and the seedbed can easily be damaged by wind erosion. Seed and soil moisture contact is critical for rapid emergence (NDSU, 2007a).

I.4.7.4. Fertilizer

Effective nutrient management and maintenance of good soil fertility is essential for high yielding and high quality canola. Nitrogen, phosphorus, potassium, sulfur, and boron, depending on the region, are the most limiting nutrients for successful canola production (Brown et al., 2008). Soil sampling and testing is the first step in assessing soil nutrient levels and the requirements for supplemental fertilizers. The availability of soil nutrients is dependent on soil acidity or the pH level and must be included in this assessment. The ideal soil pH for growing canola is between 6.0 and 7.0. Canola yields will be adversely affected when the soil pH is below 5.5 (Brown et al., 2008).

Nitrogen is the most limiting of all plant nutrients in canola and sufficient nitrogen must be available to the plant at every growth stage. Supplemental nitrogen requirements for North Dakota are based on the yield potential of canola, nitrate nitrogen available in the soil at 0-24 inch depth, and the previous crop credit for nitrogen.

Research studies have shown that nitrogen recommendations can be capped at 150 pounds per acre in the cooler, moister areas of the state without impacting yield. In the drier, warmer areas of the state nitrogen rates can be capped at 120 pounds per acre. Canola is very sensitive to fertilizer salts and no more than 5 pounds of nitrogen per acre is recommended for placement with the seed at planting on medium-textured soils (Franzen and Lukach, 2007).

Canola has a moderate requirement for phosphorus and phosphorus fertilizer rates are based on soil tests. Phosphorus is not mobile in the soil and should be banded with the seed at planting or incorporated into the soil before planting (Brown et al., 2008). A starter fertilizer rate of 20-30 pounds of P_2O_5 per acre is generally sufficient for most soil test levels unless the grower intends to build up phosphorus levels in the soil (Franzen and Lukach, 2007). Potassium requirements are also based on soil tests. Many soils contain sufficient levels of potassium requiring no potassium fertilizers. Potassium applications are not needed when the soil test indicates 160 ppm or more of potassium (Franzen and Lukach, 2007). Potassium can also be applied as a starter fertilizer with or alongside the seed at planting.

Canola has special requirements for sulfur and it is often the second most limiting nutrient in canola production (Brown et al., 2008). Sulfur deficiencies result in yellowing between leaf veins, cupped leaves and stunting (Franzen and Lukach, 2007). In addition, in the presence of sulfur deficiency flowering is delayed, seed often does not set, and pods will be barren or poorly developed. North Dakota studies have demonstrated significant yield increases from sulfur applications (Franzen and Lukach, 2007). Since soil tests tend to overestimate available sulfate and are highly variable, North Dakota specialists recommend 20 to 30 pounds of sulfur per acre when medium to low levels of sulfur are detected and 10 to 15 pounds of sulfur per acre when high levels of sulfur are detected (Franzen and Lukach, 2007). The sulfur fertilizer should be in the form of ammonium sulfate, ammonium thiosulfate or potassium thiosulfate since canola takes up sulfate sulfur.

Canola requires more boron than most other crops. Boron at 1 to 2 pounds per acre should be broadcast when the soil tests show less than 0.5 ppm boron (Brown et al., 2008). Canola has not shown yield responses to applications of micronutrients such as chlorine, copper, iron, manganese, molybdenum or zinc (Brown et al., 2008).

I.4.7.5. Harvesting

The grower's challenge is to harvest canola during the very brief optimum stage when most seeds are mature but the pods are not so dry that they will shatter. The brevity of this optimum stage is one of the factors limiting canola production (USDA-ERS, 2010b). Harvesting too early results in higher green seed content. Green seeds increase refining costs and thus reduce seed value (Brown et al., 2008). Shattered pods result in seed loss and volunteer canola in subsequent crops.

Most canola is harvested using one of two methods: 1) direct combining of standing canola, and 2) swathing followed by combining. In the latter method, the canola swath is

allowed to cure and ripen for a minimum of 10 to 14 days before harvesting which hastens maturity and avoids frost damage in areas with a short growing season (Boyles et al., 2009). Direct combining is generally recommended for winter canola in the southern Great Plains region because dry-down is accelerated by high air temperatures during seed ripening (Boyles et al., 2009).

Canola seed is sensitive to heating in storage and must be stored under cool, dry conditions (Boyles et al., 2009; NDSU, 2007a).

I.4.7.6. Irrigation and Water Use

Canola has similar moisture requirements as small grains, but is less tolerant of drought conditions. However, according to the USDA Census of Agriculture, only 13,535 or 1.2% of canola acres were irrigated in the U.S. in 2007 (USDA-NASS, 2009).

I.4.7.7. Insect Pest Management

Insect pests can reach infestation levels that cause yield reductions and often a corresponding reduction in the oil content of canola. Some aphids act as vectors for plant viruses (Brown et al., 2008). Canola seedlings are especially vulnerable to chewing insects. A severe infestation of flea beetles can completely destroy a stand of canola seedlings. A firm seedbed and adequate fertilizer will help plants outgrow the beetle damage during the seedling stage (NDSU, 2007a). Late season insect pests can typically cause yield losses of 20 to 50% in canola when left uncontrolled (Brown, et al., 2004). Damage from insect pests is more severe during periods of stress, especially drought stress. Spring and winter canola types are impacted by the same insect pests. However, some pests negatively impact one type more than the other.

Flea beetle (*Phyllotreta cruciferae*) is a serious insect pest in spring canola wherever it is grown in the U.S. (Brown et al., 2008). This insect pest is less important in winter canola unless the winter crop is planted very early or in regions where winter and spring canola is grown in the same area. An insecticide seed treatment (imidacloprid or clothianidin) may be needed in spring canola to protect canola during the seedling stage (NDSU, 2007a). Minimum or no-tillage systems generally have lower infestations of flea beetles (Weiss, et al., 2009). In addition, planting early can allow establishment of the canola prior to beetle emergence.

Insect pests causing serious damage to canola in the southern Great Plains in addition to flea beetle include grasshoppers (*Stethophyma sp.*), army cutworms (*Euxoa sp.*), diamondback moth larvae (*Plutella xylostella*) , aphids (*Myzus persicae, Lipaphis erysimi*), and root maggots (Boyles et al., 2009). Aphids have become the most important insect pest of canola in this region.

Crop rotation, controlling volunteer canola and wild mustard plants and managing crop residue are all important cultural practices for insect control.

Insect Pests and Herbicide-Tolerant Canola: In a three-year study (2000-2002) in southwestern Canada researchers compared the results of insect damage in glyphosate-

tolerant, glufosinate-tolerant and conventional canola. Insect damage from three pests, the flea beetle (*Phyllotreta cruciferae*), the cabbage seedpod weevil (*Ceutorhynchus assimilis*) and the lygus bug (*Lygus sp.*), was documented. Researchers found no differences in insect damage among the three cultivars (Cárcamo and Blackshaw, 2007).

I.4.7.8. Disease Management

Disease management is discussed in detail in Petition Section VIII.E. Plant diseases can be a serious problem in canola production. Diseases attack canola at all stages of development, can be soil borne, seed borne, or airborne and also can spread from infected crop residue (Boyles et al., 2009).

Blackleg (*Leptosphaeria maculans*) and Sclerotinia stem rot (*Sclerotinia sclerotiorum*) are serious diseases in spring and winter canola. Other diseases that affect canola include Alternaria black spot (*Alternaria spp*.), downy mildew (*Peronospora parasitica*), powdery mildew (*Erysiphe cruciferarum*), black rot (*Xanthomonas campestris),* and aster yellow (Boyles et al., 2009; NDSU, 2005). Diseases are managed primarily through use of genetics, seed treatment, chemical application and crop rotation.

Blackleg: Blackleg a fungal disease caused by *Leptosphearia maculans,* first detected in Saskatchewan in 1975, is a serious disease in the major North American canola production areas (SMA, 2009; NDSU, 2004). The blackleg fungus is present in most canola areas in North Dakota and is most common in the northern part of the state (NDSU, 2004), as it is in the western Canadian provinces. It is less common in Minnesota (Lamey, et al., 2003). Highly aggressive strains are present in Saskatchewan, Manitoba, and in some parts of North Dakota (NDSU, 2004). Blackleg was first reported in Oklahoma in 2009 (Dominiak-Olson, et al., 2009). It is not present in the Pacific Northwest (Brown et al., 2008).

Canola varieties currently available for planting in North Dakota have been bred for resistance to blackleg (Kandel, 2010). Canola seeds should be treated with a fungicide that is effective in controlling blackleg (Dominiak-Olson et al., 2009; NDSU, 2004). While the fungus can spread via spore migration from adjacent fields and from infected seeds, a study showed that most infection comes from infected residue in the field itself (NDSU, 2004). To prevent spread to a new crop, NDSU Extension specialists recommend using a four year rotation with blackleg susceptible canola (NDSU, 2005). Oklahoma State University Extension specialists recommend three to five year rotations (Dominiak-Olson et al., 2009). Long-term studies have found that 3- to 4- year rotations and genetic resistance are the most effective means to control blackleg (TopCropManager, 2011b). For crop rotations to be effective, plants that can harbor blackleg, such as volunteer canola and wild mustard, must also be controlled (NDSU, 2004).

Sclerotinia Stem Rot (SSR): Sclerotinia stem rot (*Sclerotinia sclerotiorum*) is one of the most serious and common diseases of canola in the northern Great Plains. Wet weather conditions around flowering facilitate fungal infection (Markell, et al., 2009). SSR occurs in canola, dry beans, soybeans, peas, lentils and chickpeas, but does not occur in

members of the grass family, including small grains and maize. It is also present in the Great Plains, but its impact has been minimal (Boyles et al., 2009). Crop rotation is important in managing the disease, and a preferred rotation would have canola planted after two or more nonsusceptible crops (Boyles et al., 2009; Markell et al., 2009). Many broadleaf weeds, including lambsquarters, Canada thistle, ragweed and marsh elder are also susceptible host plants (Lamey and Meronuck, 1999).

Fungal Disease and Glyphosate Use: **S**cientific evidence indicates that at recommended use rates, glyphosate-based herbicides applied in conventional or glyphosate-tolerant cropping systems do not result in increased susceptibility to diseases (Baley, et al., 2009; Lee, et al., 2000; Njiti, et al., 2003); Sanogo, et al., 2001). This observation has been reinforced through several years of commercial experience and results of grower surveys which indicate that the overall performance of glyphosate-tolerant crops, as measured by yield and constant increase in acreage, is equal to or greater than that of conventional varieties.

Scientists from Purdue University Extension recently published an article assessing whether glyphosate use has impacted crop production and disease development (Camberato, et al., 2011). Prior research from Purdue University (and research published by others) had previously found that plants sprayed with non-glyphosate herbicides were more susceptible to biological and physiological disorders, suggesting that plants already weakened by the herbicide application are more susceptible to infection. However, research found that glyphosate-tolerant soybeans and wheat are no more susceptible to soil-borne fungal diseases than conventional glyphosate-sensitive varieties, regardless of whether or not glyphosate is applied (Baley et al., 2009; Njiti et al., 2003). The use of herbicides, which have been protecting crops from weeds for more than 50 years, has not been linked to yield-limiting disease during that time (Camberato, et al., 2011).

I.4.7.9. Weeds and Weed Management

Weeds compete with canola for light, nutrients, and soil moisture and can be a major limiting factor in canola production. Canola yields can be reduced by as much as 50% due to weed competition with canola (CCC, 2006b). Weed control is discussed in detail in Petition Section VIII.F.

I.4.7.9.1. Problem Weeds in Canola – Major Production Area

Table I-3 lists weeds reported as the most common weeds in spring canola production in North Dakota, the primary U.S. canola production area.

Table I-3. Common Weeds in Spring Canola Production in North Dakota

Annual Grass Weeds	Annual Broadleaf Weeds	Biennial and Perennial Weeds
Foxtail, green and yellow	Buckwheat, wild	Quackgrass
Volunteer Cereals	Kochia	Thistle, Canada
Wild Oat	Field Pennycress	
	Lambsquarters, common	
	Mustard, wild	
	Pigweed species	
	Thistle, Russian	

Source: Zollinger, 2003; Jenks, NDSU, Personal Communication, 2010.

In addition to its high potential for causing yield losses, wild mustard is a serious seed contaminant in canola and can cause price discounts or rejection in the market. Extension advisors recommend special attention to controlling this weed prior to planting in canola fields (NDSU, 2007a).

Research studies have shown that Canada thistle is three or four times more competitive than wild oats in canola fields (CCC, 2006b). Only 10 Canada thistle plants per square meter have resulted in 10% canola yield loss, while 40 plants per square meter have resulted in over 50% canola yield loss (CCC, 2006b). An average infestation of wild oats will range from 60 to 100 plants per square meter. A wild oat density of 30 plants per square meter has caused between 15 and 20% canola yield loss (CCC, 2006b). As can be seen in Table I-7 glyphosate provides good to excellent control of both Canada thistle and wild oats.

Another weed found on canola acres, but not listed in the table above, is dandelion (TopCropManager, 2011a). Dandelion field coverage is greatest in minimum-tillage fields, and it is correlated with yield loss. For example a dandelion infestation providing 50% ground cover caused between 39% and 64% canola yield loss (University of Manitoba, 2002).

I.4.7.9.2. Problem Weeds in Canola – Minor Production Areas

Much less information specific to the impacts of weeds on canola is available for minor production areas. Boyles et al. (2009) identified the weeds in Table I-4 in canola production in Oklahoma, Kansas and Nebraska:

Table I-4. Most Common Weeds in Winter Canola in the Great Plains

Annual Grass Weeds	Annual Broadleaf Weeds	Winter Annuals
Japanese brome	Blue mustard	Henbit
Cheat	Bushy wallflower	Common chickweed
Downy brome	Wild mustard	Cheatgrass
Rescuegrass	Tumble mustard	Down bromegrass
Feral rye	Tansy mustard	Mustards
Jointed goatgrass	Flixweed	Volunteer cereals
Italian ryegrass	Field pennycrest	
Wild oat	Shepherd's purse	
Volunteer cereals		

Source: Boyles et al., 2009.

Davis (2010) and Wysocki (2010) identified the weeds in Table I-5 in canola production in the Pacific northwest:

Table I-5. Most Common Weeds in Spring and Winter Canola in the Pacific Northwest

Annual Grass Weeds	Annual Broadleaf Weeds	Perennial Weeds
Downy brome	Field pennycress	Canada thistle
Wild oat	Catchweed bedstraw	
Italian ryegrass	Prickly lettuce	
Volunteer cereals	Chamomile mayweed	
Rattail fescue	Common lambsquarters	
	Redroot Pigweed	
	Russian thistle	
	Wild mustard	
	Tumble mustard	
	Birdsrape mustard	

Source: Davis, University of Idaho, Personal Communication, 2010; Wysocki, Oregon State University, Personal Communication, 2010.

I.4.7.9.3. Weed Management

Weeds are best managed through a combination of agronomic practices also known as integrated weed management (IWM). Components of IWM in canola production can include the following (Harker, 2011; NDSU, 2007a):

Appropriate Tillage: Spring or fall pre-plant tillage can effectively reduce the competitive ability of weeds by burying the plants, disturbing or weakening the root systems or causing sufficient physical injury to kill the plants. Harrowing canola seedlings is not recommended. (NDSU, 2007a).

Careful Seeding and Fertilization: Careful planting of canola at the correct depth can help ensure a vigorous and early-emerging crop that can compete well with weeds. Placement of nitrogen fertilizer in or too close to the seed row can damage the crop (Harker, 2011). High seeding rates can also make canola more competitive. Other researchers have found that applying nitrogen in the spring instead of the fall resulted in lower weed biomass and a 20% decrease in the weed seedbank (Blackshaw, et al., 2005).

Use of Competitive Crop Cultivars: Hybrids can be strong competitors with weeds (Harker, 2011). Over 95% of the acreage in the U.S. is planted with hybrid canola varieties (Bonnetta, Monsanto Company, Personal Communication, 2010).

Early Weed Removal: Early weed removal is important to attain high canola yield (Harker, et al., 2008; Harker, 2011).

Crop Diversity and Rotation: Because different crop species and their associated management practices present different challenges for weeds, crop diversity and rotation can help prevent creating advantages for any given species (Harker, 2011).

Combining Optimal Agronomics: Researchers have demonstrated optimized weed control associated with applying multiple optimal agronomic practices such as early seeding, higher seeding rates, spring-applied fertilizer and timely herbicide applications (Blackshaw et al., 2005; Harker, 2011).

Careful Harvesting: Careful harvesting can reduce seed losses and the need to control volunteer canola in subsequent crops. Spring or fall pre-plant tillage can effectively reduce the competitive ability of weeds by burying the plants, disturbing or weakening the root systems or causing sufficient physical injury to kill the plants. (NDSU, 2007a).

As discussed in Petition Section VIII, the great majority of the canola grown in North America is herbicide-tolerant. In North Dakota in 2008, 56% of the canola was glyphosate tolerant and 39% was glufosinate tolerant (Zollinger et al., 2009).

Herbicides were applied to over 97% of the canola acres in North Dakota in 2008 (Zollinger et al., 2009). The number of registered herbicides for canola is relatively limited compared the number available for use in other crops such as maize, soybean, and small grain cereals. Ethalfluralin and trifluralin are pre-plant incorporated herbicides and clopyralid, sethoxydim, clethodim, and quizalofop are post-emergence in-crop herbicides for control of annual grasses and broadleaf weeds in conventional canola varieties (NDSU, 2005). In addition, glyphosate herbicide can be applied as a pre-plant burndown treatment for control of emerged weeds in no-tillage systems. Table I-6 summarizes the herbicides used in canola in North Dakota in 2008. Two applications of glyphosate are made on approximately 42% of the canola acreage (Table I-6). In most cases, glyphosate is commonly used as a burndown treatment to remove weeds prior to planting, and the

second application is in-crop to control weeds that emerge after planting in glyphosate-tolerant canola.

Table I-6. Herbicide Usage in Canola in North Dakota in 2008

			Number of Applications/year	
Herbicide	**Acres Treated**	**% Acres Treated**	**% of Acres Receiving One Application**	**% of Acres Receiving Two Application**
Clethodim	123,600	13.6	100.0	
Glufosinate	354,300	38.9	99.4	0.6
Glyphosate	660,300	72.6	58.0	42.1
Imazamox	21,200	2.3	48.4	51.6
Quizalofop	31,700	3.5	100.0	
Other herbicides*	29,200	3.2	100.0	

Source: Zollinger et al., 2009.
*The broadleaf herbicide clopyralid is included in the "other" category.

Table I-7 summarizes the efficacy of herbicides on weed species commonly present in canola. Glyphosate is rated excellent on all but one annual grass (wild oat – good to excellent). Glufosinate and imazamox are rated excellent or good-excellent on annual grasses except volunteer cereals and field sandbur, respectively, where they are rated fair to good. Glyphosate is the only herbicide in canola that is rated excellent on quackgrass, a perennial grass species. Glufosinate is rated excellent or good-excellent on all the annual and biennial broadleaf weeds listed while rated poor on Canada thistle. The ratings for glyphosate are more variable on broadleaf weeds ranging from excellent to poor-excellent for all annual and biennial weeds and good to excellent on Canada thistle. While imazamox is rated excellent on some broadleaf weeds it is also rated poor on buckwheat and Canada thistle. In terms of control of the main problem weed species, Canada thistle, wild mustard and wild oat, glyphosate is rated good to excellent on all; glufosinate is good to excellent for wild oat, excellent for wild mustard, but poor for Canada thistle. Of the herbicides, other than glyphosate, only clopyralid is effective on Canada thistle, but it provides no control for wild oat or wild mustard. None of the herbicides, other than glyphosate and glufosinate, control wild mustard.

Table I-7. Weed Species Responses to Herbicides in Canola[1]

Product	Foxtail, Green	Foxtail, Yellow	Volunteer Cereals	Wild Oat	Buckwheat	Kochia	Lambsquarters	Mustard, Wild	Redroot Pigweed	Thistle, Russian	Quackgrass	Thistle, Canada	Flixweed
Preplant Incorporated Only													
Ethalfluralin	E	E	G	F	P-F	F-G	E	N	E	G-E	N	N	P
Trifluralin	E	E	N	P-F	P-F	F	G-E	N	E	G	N	N	P
Postemergence													
Clopyralid	N	N	N	N	F-G	N	P-F	N	P	P-F	N	E	N
Imazamox	E	G-E	G-E	E	P	E	F	E	E	G-E	F	N-P	E
Glyphosate	E	E	E	G-E	F-G	F-E	P-E	G-E	E	G	E	G-E	G-E
Glufosinate	E	G	F-G	G-E	E	E	F-G	E	E	G-E	P	P	G-E
Quizalofop	E	G-E	E	G-E	N	N	N	N	N	N	G-E	N	N
Sethoxydim	E	E	E	G-E	N	N	N	N	N	N	F	N	N
Clethodim	E	E	E	E	N	N	N	N	N	N	G	N	N

[1]Source: NDSU, 2005. Weed control ratings based on the following scale: E = Excellent = 90 to 99% control, G = Good = 80 to 90% control, F = Fair = 65 to 80% control, P = Poor = 40 to 65% control, and N = None = no control.

I.4.7.10. Canola Volunteers

Volunteer canola is defined as a plant that has germinated and emerged unintentionally in the production of a subsequent rotational crop. Canola seeds will remain in a field after canola harvest, primarily as a result of pods shattering before or at harvest time. Researchers have estimated that an average of 3,000 seeds per square meter may be lost in harvesting (Harker, 2011). Volunteer canola will compete with the succeeding rotational crop and may affect yield depending on the density (NDSU, 2005). Although canola can volunteer for several years following a canola crop (Beckie and Warwick, 2010), problems with volunteer canola in rotations are not common (Boyles et al., 2009). Beckie and Warwick (2010), documented "the longest persistence of oilseed rape volunteers in Canada" (7 years) and concluded that their results supported the findings from previous studies that volunteer oilseed rape populations generally do not persist in the absence of replenishment of the seed bank. Researchers investigating the persistence of canola seeds in the seedbank for several different canola genotypes found, after one, two, and three winters, maximum persistence of 44, 1.4 and 0.2% of the original seedbank, respectively (Gulden, et al., 2003). In another three-year study in which wheat was planted for three years (2001, 2002 and 2003) following canola in 2000, researchers found average canola densities of 6.2, 0.7, and 0.0 plants per square meter in the first, second, and third year of the wheat crops. Researchers concluded that preventing seed production in new canola volunteers in 2001 reduced canola densities in subsequent years (2002 and 2003) below those required to mitigate weed-crop competition influences in most crops (Harker, et al., 2006).

Data from Canada suggest that presence of a herbicide tolerance trait is not a major factor influencing volunteer canola abundance (Beckie and Warwick, 2010). Beckie and Warwick (2010) also report the ranking of oilseed rape as a weed species in Canadian province field surveys went from 10th in relative abundance in the mid-1990s (when canola was mostly non-herbicide tolerant) to 12th in 2001-2003, when herbicide tolerant canola was widely used. Based on estimated crop yield loss, the economic impact of volunteer wheat and oilseed rape is ranked third and eighth, respectively, among weedy species (Beckie and Warwick, 2010).

In spring canola, seeds that remain on or near the soil surface may germinate in the fall and be killed by frost (NDSU, 2005). Researchers have concluded that producers can expect from 1 to 9% of canola harvest losses to emerge in the spring immediately following a sown canola crop and that an effective management strategy to limit long-term volunteers may be to avoid tilling in the fall and then till in the spring after the seeds have germinated (Lawson and Van Acker, 2005). In winter canola, the seed typically germinates after summer rains and can be controlled by tillage before planting the next rotational crop.

Many of the same management practices for weed management discussed in Petition Section VIII.F are applicable to control of volunteer canola including: high seeding rates, crop rotation, early weed removal (CCC, 2005) and careful harvesting.

Multiple herbicide options are available for pre-plant burndown that will control herbicide-tolerant canola prior to seeding cereal or flax (CCC, 2005), which are recommended rotational crops following canola in North Dakota. Burndown options for control of herbicide-tolerant volunteer canola are more limited prior to planting broadleaf crops such as field pea, lentil, sunflower, dry bean or chick pea (CCC, 2005).

However, volunteer canola can be controlled through herbicide applications and with appropriate rotational crop management. Table VIII-11 in the Petition provides a detailed description of the rotational cropping practices immediately following canola by state. Table VIII-12 in the Petition provides a listing of herbicides that control volunteer glyphosate-tolerant canola in the various rotational crops and their effectiveness.

I.4.7.11. Herbicide Resistance - General

Herbicide resistance is the inherited ability of a plant to survive and reproduce following exposure to a dose of herbicide normally lethal to the wild type (WSSA, 1998). For a more in-depth discussion of herbicide resistance see Appendix J.

In the mid-1950s, Harper (1957) theorized that annual, repeated use of any herbicide could lead to shifts in weed species composition within a crop-weed community. Similarly, Bandeen, et al. (1982) suggested that a normal variability in response to herbicides exists among plant species and tolerance can increase with repeated use of a herbicide. To simplify, herbicide resistance in weeds is a result of natural selection. Plants of a given species are not all identical, but are made up of biotypes with various genetic traits. Biotypes possess certain traits or characteristics not common to the entire population. Herbicides that suppress or kill weeds exert selection pressure on weed populations. When a herbicide is applied, surviving plants, those that had reduced sensitivity to the herbicide, have a competitive reproductive advantage with progeny more likely to possess the same or superior herbicide resistance. With repeated application of the same herbicide and no other herbicide or weed control practice, the resistant biotypes can become the dominant biotype in that weed community. As of May 2011, 358 herbicide resistant weed biotypes have been reported to be resistant to 21 different herbicide modes of action worldwide (Heap, 2011c). Glyphosate-resistant weeds account for approximately 6% of the herbicide resistant biotypes while weeds resistant to herbicides that inhibit acetolactate synthase (ALS) account for 31% of the herbicide resistant biotypes (Heap, 2011b; Heap, 2011a).

For as long as herbicide resistance has been a known phenomenon, public sector weed scientists, private sector weed scientists and growers have been identifying methods to address the problem. For instance, when a farmer uses multiple weed control tools, resistant biotypes generally will not become the dominant biotype within a population. By contrast, weed resistance is known to occur most rapidly in areas where there is a sole reliance on a single herbicide used repeatedly over multiple crop generations for the management of a specific weed spectrum.

When a grower encounters a biotype that is resistant to a herbicide being used, the grower needs to utilize an alternate method of weed control. Management practices that can be used to retard the development of resistance, such as those routinely used by canola growers, include herbicide mixtures, herbicide rotation, and crop rotation. The WSSA reported that weed scientists know that the best defense against weed resistance is to proactively use a combination of agronomic practices, including the judicious use of herbicides with alternative modes of action either concurrently or sequentially (WSSA, 2010).

I.4.7.12. Herbicide Resistant Weeds of Economic Importance in Canola

Herbicide resistance in weeds causing yield losses in the major U.S. canola production area are summarized in I-8 along with the herbicide mode of action (MOA).

Table I-8. Herbicide Resistant Common Weeds in Top Five U.S. Canola-Producing States in 2010

State	2010 Total Area Planted (acres)	Glyphosate Resistant Common Weeds in Canola	Resistant Common Weeds in Canola with Alternative Modes of Action
North Dakota	1,280,000	None	Wild mustard, Kochia, Wild oat, Green foxtail
Oklahoma	60,000	None	Cheat (rye brome)
Minnesota	46,000	None	Lambsquarters, Wild oat, Kochia, Yellow foxtail
Idaho	19,500	None	Prickly lettuce, Kochia, Russian thistle, Italian ryegrass, Wild oat, Mayweed chamomile
Montana	17,500	None	Kochia, Russian thistle, Wild oat

Source: USDA-NASS, 2011c; Heap, 2011d; Heap, 2011e; Heap, 2011f; Heap, 2011g; Heap, 2011h.

To date only one glyphosate resistant weed has been identified in North Dakota, common ragweed (*Ambrosia artemisiifolia*) (Heap, 2011g). Based on information from NDSU, known or suspected glyphosate-resistant weeds have been identified in soybean fields in two counties in eastern central North Dakota (Stachler and Christoffers, 2010). These counties are not major production counties for canola (USDA-NASS, 2007). In addition, glyphosate resistant populations of giant ragweed (*Ambrosia trifida*), common ragweed (*Ambrosia artemisiifolia*) and common waterhemp (*Amaranthus tuberculatus*) have been identified in Minnesota (Heap, 2011e). None of these weeds are listed as common in spring canola production fields in North Dakota (Table I-3).

Glyphosate-resistant Italian ryegrass (*Lolium multiflorum*) has been identified in Oregon (Heap, 2011b) and glyphosate-resistant horseweed (*Conyza canadensis*) has been identified in Oklahoma (Heap, 2011h).

Development of herbicide-resistant weeds is of particular concern in the scientific agricultural community. For example, Harker (2011) emphasizes the importance of integrated weed management to help preserve herbicide-tolerant technology, which has been shown to provide superior weed management, yield and environmental performance (see Appendix Section I.4.7.9.3.). In addition, the Herbicide Resistance Action Committee's provides guidelines for prevention and management of herbicide resistance (HRAC, 2009). These guidelines recommend an integrated approach to weed resistance management including crop management (i.e. row spacing, etc), cultural techniques and herbicides.

Canola is grown in rotation with crops that use herbicides other than glyphosate, and both glyphosate- and glufosinate-tolerant canola is produced. Powles (2008) concludes that where diversity in weed management systems is maintained, weed control by glyphosate can be sustainable. While glufosinate-tolerant canola is not yet available for winter canola, winter canola is grown in rotation primarily with wheat, which utilizes herbicides with alternate modes of action.

Glyphosate-resistant weeds may occur in canola fields. Some means of weed management other than glyphosate would need to be used for these weeds. Methods would be similar to those used for glyphosate-tolerant canola volunteers (Appendix Section I.4.7.10).

I.4.7.13. Crop Rotation

One of the main purposes of growing canola in rotation with other crops is to mitigate or break disease cycles (Brown et al., 2008; NDSU, 2005). As discussed in Petition Section VIII.H., canola rotation is very important for managing blackleg and Sclerotinia stem rot. Some of the other purposes of rotations are to improve or maintain soil fertility, reduce erosion, reduce the build-up of pests, reduce risk of weather-related crop damage, improve weed control and increase net profits through yield increases (Peel, 1998). Compared to continuous cereal production, including canola in small grain rotations has resulted in 17 to 20% yield increases in small grain cereals (Brown et al., 2008). Incorporating canola in small grain rotations has also decreased disease incidence and increased quality of cereal crops. Including canola in a small grain cereal rotation provides broader, more effective herbicide options for controlling certain weeds such as downy brome, jointed goat grass, wild oat, Italian ryegrass and feral rye that are difficult to control in winter wheat (Brown et al., 2008).

Table VIII-11 in the Petition provides a description and quantification of the rotational cropping practices immediately following canola, by state, that account for 99+% of the total canola acreage. For each state, the table shows quantitative data for the various crops that are rotated with canola, and also shows the percent of glyphosate-tolerant crops that are included in the rotations.

In a 2008 agronomic survey of canola growers in 11 counties representing the major canola growing areas of North Dakota and Minnesota, researchers reported that 17 of the 45 fields planted canola once in every five year rotation, with other rotations being canola every other year, every three years and every four years (Mazurek, et al., 2008).

I.4.8. Raw and Processed Agricultural Commodities

I.4.8.1. Canola as a Food Source

Canola is grown principally for its oil which is extracted from the seed, and has both food and industrial applications (Petition Section II). Canola is approximately 40% oil and 60% meal (Colton and Sykes, 1992). Canola seeds are first flaked by a rolling process in preparation for oil extraction after which the flakes are placed in a cooker. Heating reduces the viscosity of the oil and inactivates certain enzymes that can break down glucosinolates to produce isothiocyanates and nitriles which are harmful when fed to animals (Booth, 2004). After heating, canola seeds undergo mechanical extraction to produce a cake with an oil content of less than 20%, followed by solvent extraction using hexane to remove the bulk of the remaining oil. Several additional processing steps further refine the oil by removal of various undesirable constituents (Booth, 2004; Carr, 1995).

Canola oil is high quality oil that is used in a variety of foods including frying and baking oils, salad oils, margarines and shortenings, and is the most valuable component of canola seed.

Canola oil has well established heart health benefits and the U.S. FDA has issued a qualified health claim based on its ability to reduce the risk of coronary heart disease (U.S. FDA, 2006).

Recently, methods have been developed for producing a water-soluble canola/oilseed rape protein isolate having at least 90% protein that can be used in products including dairy and grain products, fruit and vegetable juices, salad dressings, meal replacements and nutritional bars, have been developed. The U.S. FDA has no objection to the GRAS notification for canola protein isolate for use in food applications (U.S. FDA, 2010) and the first commercial production took place in 2010 (BioExx, 2010).

I.4.8.2. Canola as a Feed Source

The solid residue or meal left after oil extraction of canola is used as high protein animal feed. Canola meal is used in poultry, pig, beef and dairy cattle feeds, and can also be used in aquaculture diets for salmon, catfish and trout (CCC, 2009). Canola meal contains approximately 40% protein, 13% crude fiber and essential amino acids including lysine and sulphur-containing amino acids that are most often deficient in cereal meals (Bell, 1995).

Compared to other oilseed-based feed sources, canola meal has slightly less digestible energy value. Therefore, animals with requirements for intermediate energy levels such as dairy cattle and laying chickens perform well on canola meal, while high energy animals such as broiler chickens perform better on diets with soybean meal (CCC, 2009).

I.4.9. Cross pollination and Gene Flow to Sexually Compatible Species

This section discusses the potential for gene flow within *B. napus* (intraspecific) and to closely related *Brassica* species and other members of the family Brassicaceae that may be present where canola is grown. The first part of the discussion provides information on the characteristics of *B. napus* related to gene flow and the potential for gene flow to related species based on field studies. This is followed by a discussion of the potential for gene flow from *B. napus* to specific groups of plants in the environment including other canola plants, other *Brassica* crops, canola seed production areas, other *Brassica* seed production areas, native *Brassica* species, and *Brassica* and related weeds.

I.4.9.1. Background

I.4.9.1.1. Pollen-Mediated Gene Flow and Introgression

Pollen mediated gene flow (often referred to as cross pollination) occurs when pollen of one plant fertilizes ovules of a second plant. Introgression is a multi-generation process whereby one or more genes successfully incorporate into the genome of a recipient plant. Gene flow is discussed in detail in Petition Section IX, with Table IX-3 summarizing the published literature on unassisted hybridization under field conditions with *B. napus* as the male parent.

This discussion focuses on *B. napus*, as almost all the canola grown in the U.S. is *B. napus*. *Brassica napus* is predominantly self-pollinating although interplant (plants are touching one another) cross pollination rates range from 12% to 55% with a mean of 30% (Beckie, et al., 2003). Pollen of *B. napus* is heavy and sticky (OECD, 1997) and pollen movement is primarily

by insects, such as honey bees (Thompson, et al., 1999) although wind is also responsible for some pollen movement. Most (98.8%) of pollen travels less than twelve meters from its source (Scheffler, et al., 1993) although dispersal due to pollinators may occur over greater distances at low frequency (Thompson et al., 1999).

Brassica napus produces a large amount of pollen (OGTR, 2008) which can remain viable for up to four to five days under field conditions (Rantio-Lehtimäki, 1995). This, coupled with the potential for *B. napus* pollen movement, provides the possibility for hybridization between *B. napus* and related sexually compatible species.

I.4.9.1.2. Potential for Pollen-Mediated Gene Flow and Introgression of *B. napus* to other *Brassica* Species (Including *Brassica* Vegetable Species) and Closely Related Non-*Brassica* Species

A survey of the literature provides information on the potential for hybridization (cross pollination) and introgression from *B. napus* to related species. Reports of unassisted (e.g. without human intervention or assistance) field hybridization of *B. napus* to related species provide biologically relevant information that can be useful in assessing the potential for hybridization and gene introgression under field conditions.

There are reports of hybridization under field conditions with *B. napus* as the pollen donor with six species including *B. rapa*, *B. juncea*, *B. oleracea*, *H. incana*, *R. raphanistrum* and *S. arvensis* (see Petition, Table IX-3). The species *B. rapa*, *B. juncea* and *B. oleracea* are cultivated for crop production. The other species listed, *H. incana*, *R. raphanistrum* and *S. arvensis* are not cultivated for crop production, but are found in the environment. In all cases the resulting hybrids had decreased environmental fitness evidenced by a variety of characteristics including decreased pollen viability, seed production, seedling survival, etc. when compared to parental varieties.

Brassica rapa

Brassica rapa is widespread throughout temperate North America, and may be found in areas where canola is grown (BONAP, 2010). *Brassica napus* was derived from the hybridization of diploid species, *B. rapa* and *B. oleracea* (Petition Section II.A.). *Brassica napus* and *B. rapa* have a common set of chromosomes, are known to be sexually compatible, and can form hybrids under field conditions (Bing, et al., 1996; Warwick, et al., 2003). Hybridization frequencies between *B. napus* and *B. rapa* in neighboring fields or inter-planted in large plots vary from study to study, ranging from 0% up to 69% as reviewed in Devos, et al. (2009), but can be as high as 93% (Jørgensen, et al., 1996). For example, Warwick et al. (2003) measured hybridization frequencies between these two species with *B. rapa* as the maternal parent in field experiments, including natural environments and commercial *B. napus* fields. The mean hybridization frequency was 7% in natural environmental field trials and 13.6% in the commercial fields. The first-generation hybrids between *B. napus* and *B. rapa* had reduced pollen viability, produced fewer germinable seeds, and had very low (<2%) hybrid seedling survival rates (Scott and Wilkinson, 1998; Warwick et al., 2003). Additionally, unlike the *B. rap*a parent, under field conditions, seeds of F_1 hybrids between these two species lacked seed dormancy (Jørgensen, et al., 1998).

The presence of a herbicide-tolerance trait (glufosinate tolerance) introgressed experimentally under controlled conditions from *B. napus* into *B. rapa* did not increase its survival or number of seeds per plant compared to parental *B. rapa* (Snow, et al., 1999).

Collectively, these data support the conclusion that hybridization between *B. napus* and *B. rapa* is possible under field conditions, but resulting progeny demonstrate decreased fitness and likelihood of gene introgression is low.

Brassica juncea

Brassica juncea is sparsely but widely distributed in temperate North America in cultivated and disturbed areas, and may be found in areas where canola is grown (BONAP, 2010). *Brassica juncea* is an amphidiploid derived from the hybridization of *B. nigra* and *B. rapa* (Petition Section II). *Brassica napus* and *B. juncea* have a common set of chromosomes, are known to be sexually compatible, and can form hybrids under open pollination conditions (Bing et al., 1996; Frello, et al., 1995; Jørgensen et al., 1998). As reviewed in Devos (2009), in field plots with interplanted *B. napus* and *B. juncea* interspecific hybridization frequencies were low ranging from 0.3% to 3%. First-generation hybrids between *B. juncea* and *B. napus* had reduced male and female fertility, low pollen viability ranging from 0% to 28% and low seed set (Bing et al., 1996; Frello et al., 1995). *Brassica napus* and *B. juncea* can hybridize at a low rate under field conditions, but the resulting hybrids have reduced male and female fertility significantly decreasing the likelihood of introgression.

Brassica oleracea

Brassica oleracea is distributed primarily in northeastern and midwestern states, and may be found in areas where canola is grown (BONAP, 2010). As noted above and discussed in Petition Section II.A., *B. napus* was derived from the hybridization of *B. rapa* and *B. oleracea.* Therefore it is not surprising that *B. napus* and *B. oleracea* are known to have some limited sexual compatibility under open pollination conditions (Ford, et al., 2006). Hybridization between these two species under field conditions appears to be relatively rare and introgression under field conditions is unlikely to occur (Ford et al., 2006; Wilkinson, et al., 2000).

Brassica Vegetable Species

Many common vegetables are found in the genus *Brassica*. *Brassica napus* vegetables include Swedes or rutabaga and Siberian kale. *Brassica rapa* vegetables include turnip and Chinese cabbage while *B. oleracea* vegetables include cabbage, cauliflower, broccoli, collards, kale and Brussels sprouts. Gene flow from *B. napus* canola to these vegetable species is highly unlikely but may occur. However, *B. napus*, *B. rapa* and *B. oleracea* vegetables are not considered weedy, and are generally harvested prior to flowering, preventing cross pollination, hybridization and seed formation. Thus the potential for *B. napus* gene flow and introgression into closely related vegetable species is low.

Closely Related Non-Brassica Species

In addition to those reported for *B. rapa*, *B. juncea*, *B. oleracea*, there are reports of hybridization under non-cultivation field conditions with *B. napus* as the pollen donor and

H. incana, *R. raphanistrum* and *S. arvensis* as the female parent (Petition Table IX-3). As with *B. rapa*, *B. juncea*, *B. oleracea*, in all cases these hybrids had decreased environmental fitness evidenced by a variety of characteristics including decreased pollen viability, seed production, seedling survival, etc. when compared to parental varieties. Based on field studies, hybridization between these species appears to be rare and introgression under field conditions is unlikely to occur.

I.4.9.2. Potential for Gene Flow in the Crop and Seed Production Affected Environment

I.4.9.2.1. Potential for Intraspecific Gene Flow between Canola Crops

Gene flow may occur between canola crops planted close together. Many studies have been performed and are discussed in Petition Section IX. Table I-9 is from the petition and summarizes some representative studies. As shown in the table, in general, the percentage of pollen flow and potential for cross pollination diminishes with increasing distance from the source. Canola cross pollination rates are highly variable and are influenced by experimental design, size of the pollen donor and recipient populations, variety (genotype), presence and activity of insect vectors, environmental conditions (temperature, wind speed and direction, humidity, etc.) and topography. Information on pollen movement is useful for managing pollen flow during canola breeding, seed production and for identity preservation.

Table I-9. Summary of Representative Studies of *B. napus* Cross Pollination Rates at Various Distances

Reference	Distance from Pollen Source (meters)	% Cross pollination Observed
Manasse and Kareiva, 1991; Stringam and Downey, 1982	47 137 366	2.1 1.1 0.6
Manasse and Kareiva, 1991	50 100	0.022 0.011
Scheffler et al., 1993	0 1 3 6 12 24 36 47 70	4.8 1.4 – 1.6 0.35 – 0.4 0.033 – 0.11 0.016 – 0.025 0.0 – 0.0041 0.0011 – 0.0031 0.0 – 0.00034 0.0
Morris, et al., 1994	0 0.3 0.6 3 4.6	~2.0 – 3.5[§] ~1.0 – 1.5[§] ~0.75 – 1.2[§] ~0.5 – 0.75[§] ~0.5 – 0.7[§]
Scheffler et al., 1995	200 400	0.0156 0.0038
Downey, 1999	33 66 100	0.1 – 1.5 0.0 – 0.4 0.1 – 0.4
Staniland, et al., 2000	0 2.5 5 10 15 20 25 30	0.69 0.29 0.14[φ] 0.07[φ] 0.08[φ] 0.07[φ] 0.04[φ] 0.02[φ]
Rieger, et al., 2002	100 1500 >3000	0.012 – 0.014 0 – 0.197 None detected

[§]Frequencies estimated from Figure within the publication.
[φ]Values calculated from publication.

Since the development of biotechnology-derived canola researchers often use the biotechnology-derived trait as a marker in their cross pollination studies (Beckie et al., 2003; Cai, et al., 2008; Dietz-Pfeilstetter and Zwerger, 2004; Downey, 1999; Scheffler et al., 1993; Scheffler, et al., 1995; Hommel and Pallutt, 2003). Since some cross pollination does occur at short distances,

adjacent fields with different herbicide tolerant traits would be expected to produce some seeds with both traits, which studies have confirmed (Beckie et al., 2003).

I.4.9.2.2. Potential for Gene Flow in Canola Seed Production Areas

AOSCA and state seed certification standards for canola provide isolation distances and standards for seed purity. The standards provide a guide for acceptable levels of other varieties of canola seed that might be expected to be found in certified seed based on the required seed planting isolation distances. In no case is that level 0%. For example, Colorado and Minnesota both require a seed planting isolation distance for canola hybrids of 2,640 feet from other varieties of canola and impose a standard of 1.5:10,000 (0.015%) for the ratio of plants of allowable seed of other canola varieties that can be found in certified seed (Table I-2). North Dakota has required isolation distance of 660 feet from other varieties of canola and a standard of 1:500 (0.2%) for the ratio of seed of other canola varieties (Table I-2). These standards are intended to ensure that the seed produced meets standards for seed purity and quality that are appropriate for growers' needs and meet the stated purity standards set forth in state and federal laws. However, if a seed producer wishes to produce seed with more stringent purity standards than those specified by AOSCA and the individual state certification standards, the seed producer may need to implement greater isolation distances.

I.4.9.2.3. Potential for Gene Flow between Canola and Other *Brassica* Crops

Vegetable Crops

As discussed in Petition Section IXD.3., there are many *Brassica* vegetable crops in the U.S. *Brassica napus*, *B. rapa* and *B. oleracea* vegetables are not considered weedy, and are generally harvested prior to flowering, preventing cross pollination, hybridization and seed formation.

Biofumigant Mustard

As discussed in Appendix Section I.4.4.3, biofumigant mustard (*S. alba* and *B. juncea*) is used as a green manure crop. As discussed in Appendix Section I.4.9.1.2, the potential for introgression with *B. juncea* is very low, and the potential for introgression with *S. alba* is extremely low. In addition, since biofumigant mustard is tilled under before the seeds mature (McGrath and Menasha, 2009), the potential for cross pollination is extremely low.

Industrial Oilseed Rape

The cross pollination rates shown in Table I-9 would be generally applicable to cross pollination between industrial oilseed rape and canola. As discussed in Appendix Section I.4.5.3, industrial oilseed rape is grown on very limited acreage in Washington, Oregon and Idaho, with almost all the operations in Idaho, where there are strict geographic restrictions on the proximity of industrial oilseed rape to canola. Washington and Oregon also have regulations to minimize cross pollination.

Mustard Seed (for Food)

As discussed in Appendix Section I.4.5.3, mustard seed is grown on approximately 48,000 acres, in the same general areas where canola is grown. As discussed in that section, because of similarity of disease susceptibility, it is not recommended for rotation immediately following canola. Nearly all the mustard grown in the U.S. is *S. alba*, with a very small acreage of *B. juncea*. As discussed in Petition Section IX.D.4., the potential for gene flow between *B. napus* and *B. juncea* is very low, and the potential for gene flow with *S. alba* is extremely low. Based on the extremely low potential for gene flow with *S. alba*, the relatively small number of *S. alba* acres, and the low probability of a rotation immediately following canola, cross pollination between *S. alba* mustard and canola is highly unlikely. Based on the very small acreage, the low potential for introgression and the low probability of close rotation with canola, cross pollination between canola and *B. juncea* mustard and canola is highly unlikely.

I.4.9.2.4. Potential for Gene Flow in Other *Brassica* Seed Production Areas

Vegetable Seed Production

The available information on *Brassica* vegetable seed production is detailed in Attachment 1.

As discussed in Attachment 1, the only *B. napus* vegetables grown or consumed in the U.S. are rutabaga (root) and Siberian kale (greens). Some seed companies offer *B. napus* varieties called red monarch kale and red Russian kale, which appear to be variations of Siberian kale. Therefore, the greatest possibility for cross pollination with *B. napus* canola in vegetable seed production would be with rutabaga and Siberian kale. Rutabaga seed is produced on approximately 80 acres in western Washington State in Skagit and Snohomish Counties (Attachment Table 1). Based on information from Washington State University and other information presented in Attachment 1, the 80 acres of rutabaga seed production probably accounts for at least 90% of U.S. rutabaga seed production. This rutabaga seed production area is within that part of western Washington where there are strict limitations on growing canola that are intended to prevent any cross pollination with related *Brassica* species (Appendix Section I.4.6). We were unable to find any information specifically on Siberian kale seed production in the U.S., although a seed producer in the Yuma Valley of Arizona produces seeds for red monarch and red Russian kale greens (Attachment 1). Based on USDA census data, no canola is grown in Arizona; therefore, there is no potential for cross pollination from canola with these seed crops.

For the other *Brassica* and related vegetables, Table I-10 summarizes the potential for hybridization and introgression with canola, based on the information presented in Petition Section IX and summarized in Appendix Section I.4.9.1.

Table I-10. Cross Pollination Potential for Canola with Related Vegetable Seed Species

Species	Common Names	Potential for Hybridization	Potential for Introgression
B. rapa	Chinese cabbage, pak choi, choi sum, turnip, Chinese mustard, broccoli raab, mizuna, mibuna, komatsuna, tai soi (most of these are greens)	High (0-70%)	Low
B. juncea	Greens: red giant mustard, sawtooth mustard, Osaka purple mustard, southern giant curled mustard, Florida broadleaf, leaf mustard	Low (0.3 to 3%)	Very low
B. oleracea	Cabbage, broccoli, kale, cauliflower, Brussels sprouts, kohlrabi, collards, Chinese kale	Low (0.1%)	Very low
R. raphanistrum	Radish, daikon	Very low (< 0.003%)	Extremely low

Seed production of other *Brassica* and related species occurs almost entirely in areas with prohibitions or strict limitations on canola production and transport (eastern Washington, western Washington, the Willamette Valley in Oregon and Treasure Valley in Idaho), or in areas where canola production is either negligible or non-existent (California and Arizona; Appendix Section I.4.5; Attachment 1).

Aside from these regulatory and geographic features that currently minimize cross pollination, the vast majority of *Brassica* and related seed production is *B. oleracea* and *R. raphanistrum*, which have, respectively, very low and extremely low potential for introgression with *B. napus*. In greenhouse and field experiments, (Quinn, 2010) found that canola (*B. napus*) cross pollinated with a *B. rapa* vegetable species (Chinese cabbage) but did not cross pollinate with a *B. oleracea* vegetable species (broccoli). The study evaluated only cross pollination potential and not introgression. The field studies were done under a "worst case scenario" of placing the vegetable seed receptor plant in the middle of a canola field during peak flowering of both species (Quinn 2010).

The Willamette Valley Specialty Seed Association (WVSSA) seed production isolation guidelines for vegetable seed production are established to minimize the potential for cross pollination of *B. napus* canola with related vegetable species (WVSSA, 2008).

I.4.9.3. Potential for Gene Flow from Canola to Species in the Natural Affected Environment

I.4.9.3.1. Potential for Gene Flow from Canola to Native *Brassica* Species

None of the 19 *Brassica* species in the USDA plant database are native to North America (USDA-NRCS, 2010). Therefore, there is no potential for gene flow to native *Brassica* species.

I.4.9.3.2. Potential for Gene Flow from Canola to Brassica and Related Weed Species

Based on the USDA Plant Database (USDA-NRCS, 2010), the *Brassica* species that have been specifically identified as weeds in the U.S. are *B. rapa* (birdsrape or field mustard). *Brassica tourneforti* (Asian mustard), *B. juncea* (Indian mustard) and *B. nigra* (black mustard). Related species identified as weeds include *R. raphanistrum* (wild radish) and *S. arvensis* (wild mustard). The USDA plant database (USDA-NRCS 2010) shows distributions in the U.S. for these *Brassica* and related weed species as follows:

- Indian mustard (*B. juncea*) – throughout most of the U.S. and Canada
- Field mustard or birdsrape (*B. rapa*) – throughout U.S. and Canada
- Black mustard (*B. nigra*) - throughout most of the U.S. and Canada
- Asian mustard (*B. tourneforti*) – CA, NV, AZ, NM, TX
- Wild radish (*R. raphanistrum*) - throughout most of the U.S. and Canada
- Wild mustard (*S. arvensis*) - throughout the U.S. and Canada

Based on the information from Petition Section IX, the likelihood of introgression is low with *B. rapa* and very low to extremely low with the other related weed species on this list.

Introgression, i.e., the stable incorporation of genes from one differentiated gene pool into another, of a biotechnology-derived trait from *B. napus* to *B. rapa* has been demonstrated on a single occasion under commercial field conditions (Warwick, et al., 2008). Populations of *B. rapa* located close to commercial fields of glyphosate-tolerant *B. napus* were monitored for multiple years. The number of hybrids with the glyphosate-tolerance trait declined drastically from the first to fifth year of monitoring, but persisted at low levels at one of the two sites. A single glyphosate-tolerant diploid individual with 29% pollen viability was discovered five years after the last planting of glyphosate-tolerant *B. napus* confirming the presence of the herbicide tolerance trait over time (Warwick et al., 2008).

Warwick et al. (2008) noted that they had no data to suggest that the presence of a herbicide-tolerant trait in a weedy species poses a specific risk and that the trait would have positive selection value only in the presence of the herbicide. However, the selection potential of herbicide application could extend beyond the boundary of the field because of the potential advantage that drift may confer to weeds that have cross pollinated with herbicide-tolerant canola (Londo, et al., 2010).

While *Brassica* and related weeds can be found in canola, no instances or reports of introgression of traits from herbicide-tolerant canola with weeds have been found in the literature or in information from university extension offices and industry sources other than that reported by Warwick et al. (2008). To date, herbicide-tolerant canola has proved a useful tool in controlling weeds of *Brassica* and related species. For example, as discussed in Appendix Section I.4.7.9, wild mustard (*S. arvensis*) is a problem weed in canola in North Dakota, as it is in the Canadian provinces. The NDSU recommends that conventional canola not be planted on fields with heavy infestations of wild mustard. However, glyphosate, glufosinate and imazamox tolerant canola

can all be planted on heavily infested wild mustard fields (NDSU, 2007a) to achieve good weed control.

I.4.10. Ruderal Canola

Canola is sometimes found growing in locations where it was not intentionally planted. When that occurs in an agricultural field, it's called volunteer canola (Appendix Section I.4.7.10). When it occurs outside an agricultural field, the term ruderal canola applies. Ruderal canola could potentially be a weed control issue if it was invasive and/or hard to control. This section addresses these issues and also assesses whether herbicide-tolerance has affected the survivability of ruderal canola. A literature review is included in Table IX-1 of the Petition and results are summarized below.

Locations of Ruderal Canola

Canola seeds are very small and round, and can spill from trucks and rail cars during transport. Therefore, most ruderal canola is found in disturbed areas along roadways and railroads where canola has been transported (Aono, et al., 2006; Crawley and Brown, 2004; Knispel, et al., 2008; Nishizawa, et al., 2009; Pivard, et al., 2008; Saji, et al., 2005). Crawley and Brown (2004) reported that the plants typically grow within one meter of the road. Nishizawa et al. (2009) reported canola in sidewalk cracks and flowerbeds; however, this was all within a one-to three meter wide monitoring zone immediately adjacent to the roadway pavement. Some ruderal canola is found along the edges of canola fields (Knispel et al., 2008; Pivard et al., 2008).

Persistence of Ruderal Canola

Most researchers have concluded that ruderal canola populations are not self-sustaining. In a ten-year (1993 to 2003) study of 3,658 quadrants (each 100 meters long) along the edges of roadways near London, Crawley and Brown (2004) found that one percent of the quadrants were occupied (had one or more canola plants) for the entire 10-year period, but none of these had constant population density. The most frequent pattern observed was for quadrants to have canola plants present for one or two years out of ten. Canola plants outside agricultural fields can produce seed (Crawley and Brown, 1995; Knispel et al., 2008) but this is often prevented because most plants do not survive to maturity. This is due to competition from other vegetation (Crawley and Brown, 1995), management operations such as roadside mowing, the use of broadleaf herbicides, animal predation, diseases and environmental conditions (Crawley and Brown, 1995; Knispel et al., 2008; Norris and Sweet, 2002; Yoshimura, et al., 2006).

Herbicide-Tolerant Traits and Ruderal Canola

Frequency of occurrence of biotechnology-derived plants in ruderal areas was similar to the proportion of the canola area planted with herbicide-tolerant canola varieties in recent preceding years (Yoshimura et al., 2006). Biotechnology-derived canola populations have not been found to be more invasive or more persistent than conventional canola populations (Crawley, et al., 2001). The viability of the large majority of canola seed in soil declines over time (Gulden et al., 2003; Gulden, et al., 2004; Hails, et al., 1997) and biotechnology-derived canola seed has not been demonstrated to persist longer than conventional canola seed (Gruber, et al., 2004; Hails et al., 1997). Populations of glyphosate-tolerant ruderal canola can be found along roadsides.

Although glyphosate is frequently the herbicide of choice for roadside weed control (Powles, 2008), there are multiple other herbicide and mechanical options that are available to control ruderal canola (OGTR, 2010; Gover, 2011).

I.4.11. Public Health

I.4.11.1. Canola Oil

As discussed in Petition Section II.A., the primary food use of canola is canola oil, which has well established heart health benefits. According to the OECD (2001) canola oil contains one key toxicant, erucic acid. A key toxicant is a potentially significant toxic compound known to be inherently present in the species, in this case, *B. napus* (OECD, 2001). Canola oil, which by definition has less than 2% of its fatty acid as erucic acid has been affirmed as GRAS by U.S. FDA. The Codex Standard for Named Vegetable Oils (Codex Alimentarius, 2005) also specifies that canola oil cannot contain more than 2% erucic acid. In addition the U.S. FDA has no objections to the recent GRAS notification for canola protein isolate.

Canola oil derived from the current glyphosate-tolerant canola RT73 has been shown to be compositionally equivalent to canola oil derived from conventional canola (Nickson and Hammond, 2002). As noted by the National Research Council (NRC), unexpected and unintended compositional changes arise with all forms of genetic modification, including both conventional breeding and genetic engineering (NRC, 2004). However, the NRC also noted that no adverse health effects attributed to genetic engineering had been documented in the human population. Reviews on the nutritional quality of biotechnology-derived foods have generally concluded that there are no significant nutritional differences in conventional versus biotechnology-derived plants for food or animal feed (Faust, 2002; Flachowsky, et al., 2005).

I.4.11.2. Pesticide Use and Worker Safety

As discussed in Appendix Section I.3.1, the U.S. EPA regulates herbicides under FIFRA and established tolerances for herbicide levels on food, for protection of human and animal health. The U.S. EPA also regulates other pesticides, including insecticides and fungicides, that are used on canola and establishes application rates that are deemed not to have an unreasonable adverse effect. The herbicides, insecticides and fungicides typically used on canola are discussed in Petition Sections VIII.E and VIII.F. This discussion focuses on glyphosate, as MON 88302 is tolerant to glyphosate. Glyphosate has been widely used in canola fields since 1999 when the first generation Roundup Ready canola RT73 was launched. The total rate of glyphosate in-crop application to Roundup Ready canola RT73 is 0.78 lb a.e. per acre. With MON 88302 growers will have the option to make applications at 1.55 pounds a.e. per acre of glyphosate in-crop. The rates for MON 88302 are comparable to the rates used for maize and soybean (approximately 2 pounds a.e. per acre of glyphosate in-crop).

Glyphosate is a herbicide approved for use (registered) by the U.S. EPA for the control of weeds that would interfere with the growth of many food and non-food crops, including biotechnology-derived crops, as well as for control of weeds growing in non-crop areas. Currently over 70% of canola acres in the major U.S. canola producing region are treated with glyphosate as a component of a weed control regimen either in-crop or pre-plant. A more detailed discussion on

the potential impact of glyphosate on human health and the environment may be found in Appendix K.

According to the Reregistration Eligibility Decision (RED) document for glyphosate (U.S. EPA, 1993), glyphosate is of relatively low oral and dermal acute toxicity. For this reason, glyphosate has been assigned to Toxicity Categories III and IV for these effects (i.e., Toxicity Category I indicates the highest degree of acute toxicity, and Category IV the lowest). An acute inhalation study was waived by the U.S. EPA because glyphosate is a non-volatile solid, and the studies conducted on the end-use product formulation are considered sufficient (U.S. EPA, 1993). Expert toxicological reviews the World Health Organization (WHO-FAO, 2004) are in agreement that glyphosate does not pose any human acute exposure concerns for dietary exposures and thus negated the need to establish an acute reference dose.

Based on the toxicity of glyphosate and its registered uses, including use on glyphosate-tolerant crops, the U.S. EPA has concluded that occupational exposures (short-term dermal and inhalation) to glyphosate are not of concern because no short-term dermal or inhalation toxicity endpoints have been identified for glyphosate (U.S. EPA, 2006a; U.S. EPA, 2006b).

Additional evidence to support the U.S. EPA conclusion can be found in the Farm Family Exposure Study (Acquavella, et al., 2004), a biomonitoring study of pesticide applicators conducted by independent investigators. This biomonitoring study determined that the highest estimated systemic dose of glyphosate for applicators as the result of routine labeled applications of registered glyphosate-based agricultural herbicides to crops, including glyphosate-tolerant crops, was approximately 400 times lower than the RfD established for glyphosate. Furthermore, investigators determined that 40% of applicators did not have detectable exposure on the day of application, and 90% of the applicators had an estimated systemic dose of glyphosate less than 0.06% the RfD (Acquavella et al., 2004).

The biomonitoring study also found little evidence of detectable exposure to individuals on the farm who were not actively involved in or located in the immediate vicinity of labeled applications of glyphosate-based agricultural herbicides to crops. Considering the similarity of the use pattern and application rates of the glyphosate products in this study compared to those registered for use on glyphosate-tolerant crops, bystander exposure attributed to the use of glyphosate on glyphosate-tolerant crops is expected to be negligible. Therefore, the use of currently registered products containing glyphosate in accordance with the labeling will not pose unreasonable risks and adverse effects to humans or the environment. In general, the herbicidal activity of glyphosate is due primarily to a metabolic pathway that does not occur in humans or other animals, and thus, this mechanism of action is not directly relevant to the human health risk assessment. The U.S. EPA considers glyphosate to be of low acute and chronic toxicity.

Glyphosate is not considered a carcinogen; it has been classified by the U.S. EPA as a Group E carcinogen (evidence of non-carcinogenicity for humans) (U.S. EPA, 1993).

I.4.12. Animal Health

As discussed in Petition Section II.A., canola meal is a high protein animal feed. Canola is by definition low in erucic acid and low in glucosinolates, and canola meal has a history of safe use

as an animal feed source. Although canola can be used as a forage crop, the use is limited because of its potential high levels of sulfate, and potential to cause bloat and other health problems in livestock (NDSU, 2008).

The OECD (2001) identifies one key toxicant in canola meal, glucosinolates. Glucosinolates are organic compounds that contain both sulfur and nitrogen, are found abundantly in plants of the *Brassica* genus and are responsible for the pungent or biting flavors found in closely related *Brassica* species such as mustard and horseradish. The low levels of glucosinolates in canola as compared to other oilseed rape make the meal safe for use as animal feed. The standard for glucosinolates in canola is 30 μmoles/g (OECD, 2001).

Canola meal from biotechnology-derived canola has been shown to be as safe and nutritious for animals as canola meal from conventional canola (Nickson and Hammond, 2002; Taylor, et al., 2004). In a study of the effects on pigs of canola meal derived from glyphosate-tolerant canola compared to meal derived from the non-biotechnology-derived parent, researchers concluded that the CP4 EPSPS protein has no effect on growth performance, carcass characteristics, or pork quality (Caine, et al., 2007).

I.4.13. Animal, Plant and Microbial Communities Including Threatened and Endangered Species

This section discusses animal and plant communities that may be affected by canola production. Because MON 88302 is expected to serve as an alternative to Roundup Ready canola, RT73, it also discusses the affected environment of animal and plant communities as they relate to current, widespread glyphosate application on canola fields.

As a part of the reregistration evaluation under FIFRA, the U.S. EPA conducted an ecological assessment for glyphosate. This assessment compared the results from toxicity tests with glyphosate conducted with various plant and animal species to a conservative estimate of glyphosate exposure in the environment. In the Reregistration Eligibility Decision (RED) for glyphosate (U.S. EPA, 1993), the exposure estimates were determined assuming an application rate of 5.0625 pounds a.e. glyphosate per acre,[36] which exceeds the maximum labeled use rate for a single application for agricultural purposes. In its analysis, the U.S. EPA used the 5.0625 pounds a.e.per acre rate to calculate the estimated environmental concentrations (EECs) for aquatic plants and animals. Based on this assessment, the U.S. EPA concluded that effects to birds, mammals, fish and invertebrates are minimal (U.S. EPA, 1993).

I.4.13.1. Animal Communities

The affected environment for growing canola plants can generally be considered the agroecosystem (managed agricultural fields) plus adjacent areas extending beyond the intended plantings that might be affected by agricultural operations. Mammals and birds, including migratory mammals and birds, may seasonally consume seeds from the planted fields, and invertebrates can feed on the plant and surrounding vegetation during the entire growing season.

[36] Although the EPA (1993) refers to the maximum application rates as a.i. (active ingredient), it can be determined that the maximum rate used in the calculations of the EECs was in a.e. (acid equivalents).

Rodents and other small animals may inhabit canola fields, and the raptors, snakes and other animals that may prey on them are part of the affected environment. Deer may also browse in canola fields on the forage. Fish and other aquatic organisms in streams draining agricultural fields are also part of the affected environment.

Glyphosate is practically nontoxic to slightly toxic to birds, freshwater fish, marine and estuarine species, aquatic invertebrates and mammals and practically nontoxic to honey bees (which are used to assess effects on nontarget insects in general) (U.S. EPA, 1993). Glyphosate has a low octanol-water coefficient, indicating that it has a tendency to remain in the water phase rather than move from the water phase into fatty substances; therefore, it is not expected to accumulate in fish or other animal tissues.

I.4.13.2. Plant Communities

The affected environment for growing canola plants can generally be considered the agroecosystem (managed agricultural fields) areas as well as adjacent areas extending beyond the intended plantings that might be affected by agricultural operations. Plants, extraneous to the crop, which grow in planted fields can be considered weeds and are dealt with in a separate section in this document. Plants not growing in a field amongst the canola would include those in ditches, hedge rows, fence rows, wind breaks, yards, etc.

Glyphosate is a non-selective herbicide with post-emergence activity on essentially all annual and perennial plants and has the potential to impact nontarget plants as a result of runoff or spray drift (U.S. EPA, 1993). Regarding runoff, glyphosate binds strongly to agricultural soils and has low potential to move offsite dissolved in water (U.S. EPA, 1993). Moreover, glyphosate is not taken up from agricultural soils by plants. Therefore, any potential effects to non-target plants from glyphosate use are only attributed to spray drift. During the re-registration process in 1993, additional data on terrestrial nontarget plants were requested by the U.S. EPA. These additional data have been utilized in conjunction with an exposure assessment to further understand the potential risk to nontarget and threatened and endangered plants from the use of glyphosate herbicides in agriculture. Using the methodology described in a recent U.S. EPA effects determination for glyphosate (U.S. EPA, 2008b), it can be determined that there is minimal risk to terrestrial plants that are not listed as threatened or endangered at the maximum glyphosate single application rate for ground applications (3.75 pounds a.e. glyphosate per acre), and at rates just below the maximum single aerial application rate (1.48 pounds a.e. glyphosate per acre compared to 1.55 pounds a.e. glyphosate per acre).

I.4.13.3. Soil Microorganisms

Microbial populations and associated biochemical processes are critical to maintaining soil health and quality. The occurrence and abundance of soil microorganisms are affected by 1) soil characteristics like tilth, organic matter, nutrient content, and moisture capacity, 2) typical physico-chemical factors such as temperature, pH, and redox potential, and 3) soil management practices. Agricultural practices such as fertilization and cultivation may also have profound effects on soil microbial populations, species composition, colonization, and associated biochemical processes (Buckley and Schmidt, 2001; Buckley and Schmidt, 2003). Consequently, significant variation in microbial populations is expected within and among

agricultural fields. A recent study performed on the effects of glyphosate-tolerant canola on soil microbial biomass, functional diversity and enzyme activity (Lupwayi, et al., 2007) found that effects on soil microorganisms were minor and inconsistent over a wide range of growing conditions and crop management.

The effects of glyphosate and glyphosate-based formulations on soil microorganisms have been extensively investigated (Cerdeira and Duke, 2006; Sullivan and Sullivan, 2000). Results of standardized tests with glyphosate formulations performed for submission to regulatory agencies indicate no long-term effects on microorganisms in soil even at rates that exceed maximum use rates (up to five times the labeled rate). In addition, independent researchers have reviewed numerous laboratory and field studies, investigating the effects of glyphosate on soil bacteria and fungi (Felsot, 2000; Giesy, et al., 2000) and have concluded that glyphosate has a "remarkable level of safety for virtually all organisms tested". In a recent greenhouse investigation, Arango (2009) evaluated the effects of multiple glyphosate applications on the rhizosphere bacterial community associated with glyphosate-tolerant soybean. While subtle, transient shifts in community structure were noted after glyphosate applications, effective resilience and no reduction in bacterial diversity were observed for the bacterial community associated with roots of glyphosate-treated versus unsprayed glyphosate-tolerant soybean. Although some laboratory tests have shown effects on nitrogen-fixing bacteria (Moorman, et al., 1992; Santos and Flores, 1995) and soil fungi (Busse, et al., 2001; Estok, et al., 1989), effects are typically observed only under artificial laboratory conditions and at glyphosate concentrations well above normal field application rates. Several researchers have concluded that it is difficult to extrapolate results from the laboratory to the natural soil environment (Busse et al., 2001; Wan, et al., 1998; Wardle and Parkinson, 1990a; (Wardle and Parkinson, 1990b).

Investigations by Haney, et al., (2000; 2002) related to the increased use of glyphosate-tolerant crops indicate that glyphosate was degraded over time by soil microbes, even at high application rates, without adversely impacting soil microbial activity. In addition, results from field studies that have evaluated the fungal component of the soil microbial community indicate that glyphosate treatment had no deleterious effects on beneficial soil fungi (Araujo, et al., 2003; Biederbeck, et al., 1997; Busse et al., 2001; Wardle and Parkinson, 1990a; Wardle and Parkinson, 1990b). In a 4-year field study, Powell, et al. (2009) assessed effects of glyphosate applications on soil food web properties and crop litter decomposition in a glyphosate-tolerant soybean and maize rotation. The researchers concluded that: "Permanent responses in soil biota were not observed, suggesting a high level of resilience in the soil biota and a lack of a persistent effect resulting from the GM cropping system." Furthermore, Liphadzi, et al. (2005) observed that the soil microbial and nematode community was similar when glyphosate or conventional herbicides were applied to crop rotations of glyphosate-tolerant soybean and maize cultivars. Moreover, the history of safe use and yield data obtained for nearly 15 years of glyphosate-tolerant crop production, combined with in-crop applications of glyphosate-based agricultural herbicides, reinforce the findings that soil microbes and microbially mediated processes are not adversely impacted by field-rate applications of glyphosate.

I.4.13.4. Threatened and Endangered Species

Plants or animals that may inhabit areas adjacent to agricultural fields and that U.S. Fish and Wildlife Service (FWS) has identified as threatened or endangered under the Endangered Species

Act may be part of the affected environment. No *Brassica* species in the U.S. are considered to be threatened or endangered. No *Brassica* species are utilized uniquely by threatened or endangered species for survival.

Like other animals and plants, threatened and endangered species that are present near canola fields could potentially be affected by runoff from the fields and by herbicide drift. As discussed in Appendix Section I.4.13.2, for glyphosate runoff is not a concern but herbicide drift may be.

Detailed information on the potential impacts to plant and animal communities (including threatened and endangered species) by glyphosate use on glyphosate-tolerant crops is included in the USDA APHIS Final Environmental Impact Statement (FEIS) on the Deregulation of Glyphosate-Tolerant alfalfa. The information is presented in Chapter IV.C and Appendix N of the Alfalfa FEIS and is incorporated in this EA by reference, and summarized below.

As documented in the Glyphosate Tolerant Alfalfa FEIS (USDA-APHIS, 2010), spray drift is one of the pathways of concern for non-target plants; therefore, Monsanto prepared an analysis of the risk to TE species to evaluate the impacts to plants and animals from the use of glyphosate-based herbicides in conjunction with glyphosate-tolerant plants. The complete analysis was submitted to APHIS and has been reviewed by APHIS scientists to support the petition for deregulation of glyphosate tolerant alfalfa. The conclusion from the APHIS review is that threatened or endangered terrestrial or semi-aquatic plant species are not at risk from ground applications of glyphosate at rates less than 3.5 lbs a.e. glyphosate per acre, or from aerial applications at rates less than 0.70 lb a.e. glyphosate per acre. To address this issue, Monsanto has developed a web-based mitigation program called Pre-Serve that describes location-specific mitigation measures that must be implemented when glyphosate is applied at a ground application rate of 3.5 lbs a.e. glyphosate per acre or more, or an aerial application rate of 0.7 lb a.e. glyphosate per acre or more Monsanto's Technology Use Guide (TUG), which is a contractual part of its licensing agreement with growers, requires growers to access the Pre-Serve website and follow the instructions when their glyphosate ground application rate is 3.5 lbs a.e. glyphosate per acre or more or their aerial application rate is 0.7 lb a.e. glyphosate per acre or more (Monsanto Company, 2010). Only a very small percentage of glyphosate applications will require implementation of management practices. This is because the vast majority of U.S. cropland is not in close proximity to threatened and endangered plant species.

I.4.14. Physical Environment

I.4.14.1. Land Use

The total U.S. acreage of canola increased more than 200% from 367,000 acres in 1996 to 1.1 million acres in 1998, coinciding with passage of the 1996 Federal Agriculture Improvement Act. [37] Canola acreage peaked at 1.55 million in 2000 and has remained between 0.8 and 1.6 million acres since 2005 (Figure I-3).

[37] The 1996 Federal Agriculture Improvement Act, 7 U.S.C. § 7201 *et seq.*, gave growers almost complete flexibility in selecting the crops they could plant.

The acreage of glyphosate-tolerant canola increased rapidly following commercial introduction in 1999 and has remained between 50 and 70% of planted canola acres since 2001. Fluctuations in total canola acreage before and after glyphosate-tolerant canola was commercialized indicates that factors unrelated to the availability of the glyphosate-tolerant trait play a larger role in acres planted than the availability of the glyphosate-tolerant trait.

In 2008 herbicide-tolerant canola varieties were planted on approximately 95% of the canola acreage in North Dakota (Zollinger et al., 2009). Glyphosate was applied to approximately 73% of the canola acreage in 2008 which includes pre-plant burn down and in-crop applications. Glufosinate-tolerant canola was introduced in 1995 and is tolerant to post-emergence applications of glufosinate. Glufosinate is the second most commonly applied herbicide used in canola (38% of acres) in 2008.

As canola cropland has increased, production of wheat, the main rotational crop for canola, has decreased. Future increases in canola production are likely to result from canola replacing some wheat rotations in wheat monocultures or cropping systems with wheat as the main crop (Petition Section VIII.H.). Greater increases in production could result if canola is used more widely for biodiesel. Canola may be a better candidate for biodiesel in Canada and some European Union countries, where canola production is higher and soybeans are not grown on significant acreage. In the U.S., soybean oil is currently substantially less expensive than canola oil and the USDA ERS has concluded that this reduces the potential of canola as a biodiesel crop (USDA-ERS, 2010b)

I.4.14.2. Air Quality and Climate Change

Many agricultural activities affect air quality including tillage, traffic and harvest emissions, and nitrous oxide emissions from the use of nitrogen fertilizer. These agricultural activities individually have potentially adverse environmental impacts on air quality and climate and may be impacted positively or negatively by changes in agricultural practices. Issues of concern include, but are not necessarily limited to, atmospheric emission of carbon dioxide, nitrogen oxide, sulfur oxide, and particulate matter. Agricultural practices have the potential to directly and indirectly impact air quality and to contribute emissions which could lead to climate change.

Tillage contributes to the release of greenhouse gases (GHG) because of the loss of carbon dioxide to the atmosphere and the exposure and oxidation of soil organic matter (Baker, et al., 2005). Emissions released from agricultural equipment (e.g., irrigation pumps and tractors) include carbon monoxide, nitrogen oxides, particulate matter, and sulfur oxides. Nitrous oxide may also be released following the use of nitrogen fertilizer. Agriculture, including land-use changes for farming, is responsible for an estimated 17 to 32% of all human-induced GHG emissions. Herro (2008) proposes that if agriculture practices were modified, significant reductions in the release of GHGs could be achieved.

I.4.14.3. Surface Water and Groundwater Quality

Tillage and Water Conservation

Tillage practices in canola production are discussed in Appendix Section I.4.7.2. The USDA Economic Research Service defines conservation tillage as cultural operations that maintain at

least 30% cover of the soil surface by plant residue at the time of planting (Anderson and Magleby, 1997). Conservation tillage can encompass a range of management practices, from no-till to ridge- and strip-till cultivation to minimum tillage systems that restrict equipment traffic to dedicated zones. Special tillage field equipment can often perform the equivalent functions of several standard implements, reducing the necessity for multiple passes through the field. No-till is defined by USDA ERS (Anderson and Magleby, 1997) to be those practices that leave the soil undisturbed, and thus leave substantial crop residues on the surface of the planted field. Implementing conservation tillage practices can lead to both economic and production quality benefits, as well as having positive environmental impacts (www.nrcs.usda.gov). Use of conservation tillage compared to use of conventional tillage in many soils may allow 10 to 40% greater water infiltration into soils (Hoeft, et al., 2000a; Hoeft, et al., 2000b). Crop residues established by conservation tillage on soil surfaces slow water runoff, increase porosity by increasing numbers of wormholes and by means of remnants of crop residue, and reduce evaporation through the insulating ability of surface mulches. No tillage alone, without additional erosion control measures, reduced runoff and sediment loss by approximately 85% when compared with non-conservation chisel plow tillage (Zhou, et al., 2009), and no till can reduce runoff volume 35 fold compared to conventional tillage (Gregory, et al., 2005). Researchers have found significant increases in conservation tillage with the introduction of herbicide-tolerant canola (Smyth, et al., 2010).

Water Quality

Surface water may be impacted from canola production by runoff from canola fields that carries soil particles and herbicides or other pesticides to streams, rivers, lakes, wetlands and other water bodies. As discussed below, based on existing data, the soil component of runoff is a much more important contributor to surface water impacts than is the pesticide component.

Tillage causes widespread soil disturbance. Thus, erosion, topsoil loss and the resulting sedimentation and turbidity in streams are likely to increase with increased tillage. In 2009, based on the states' water quality reports, the U.S. EPA identified sedimentation and turbidity as two of the top 10 causes of impairment to surface water in the U.S. in general; in 2007, the U.S. EPA identified sedimentation/siltation as a leading cause of impairment to rivers and streams (U.S. EPA, 2007; U.S. EPA, 2009). Although a comprehensive data set has not yet been developed to prove the point, the U.S. EPA has projected conservation tillage to be the major soil protection method for improving surface water quality (U.S. EPA, 2002). The U.S. EPA identifies conservation tillage as the first of its CORE four agricultural management practices for water quality protection (U.S. EPA, 2008a).

Based on the states' water quality reports to the U.S. EPA, which the EPA makes available through its National Assessment Database, pesticides in general and herbicides in particular are a relatively minor contributor to impairment of surface water in the U.S., compared to sedimentation/siltation and turbidity (U.S. EPA, 2007; U.S. EPA, 2009). Pesticides accounted for less than one percent of reported causes of surface water impairment in all but three of the nine leading U.S. canola-producing states. In those three states, pesticides accounted for three to ten percent of reported causes of impairment. Of the pesticides that were reported as contributing to impairment among the nine leading canola-producing states, almost all are

previously used, highly persistent chemicals that are no longer registered for use in the U.S. (EPA 2008a).

I.4.15. Economics

The affected trade economic environment is defined as those countries with which the U.S. engages in canola feed, seed and food trade. The affected domestic environment is defined as any land in the U.S. that is currently producing crops that could incorporate a canola rotation, as well as land that could be converted from inactive cropland to active cropland, and land currently in the Conservation Reserve Program (CRP) that could be removed from the program and farmed. CRP is a voluntary program sponsored by the USDA offering annual rental payments over a 10-year contract period, as well as cost-share assistance, to producers establishing specific types of plant cover on marginal farmland. The affected environment for economics is primarily the farming communities where canola is grown. Production costs for canola are discussed in Appendix Section I.4.7.1.

The U.S. produced 1.9% (USDA-NASS, 2011c) of the world's 60.6 million metric tons of canola in the 2010 growing season (USDA-FAS, 2011c), and imported 0.7 million metric tons from Canada (Statistics Canada, 2011).[38] Total global U.S. imports of canola oil continue to increase steadily from 0.5 million metric tons in 2000 (USDA-FAS, 2011a) to 1.2 million metric tons in 2010 (USDA-FAS, 20111b). Although canola production in the U.S. has increased dramatically since the 1980s, it has always been far short of demand (Figure I-2). The U.S. shortfall is made up with imports primarily from Canada. In 2010 canola was planted on approximately 1.45M acres in the United States producing 1.1 million metric tons of canola, with a value of approximately $487 million (USDA-NASS, 2011a; USDA-NASS, 2011b). In a 2010 dollar-value comparison with other major crops, canola represents 0.7% of the value of the maize crop, 1.3% of the value of the soybean crop and 3.7% of the value of the wheat crop (USDA-NASS, 2011b).

Canola has been able to benefit from the same price subsidies as other commodity crops, strengthening the profit potential of this crop. In most side-by-side tests, canola has provided a greater net profit than wheat, though ease of marketing and variability in yield are off-setting factors.

Researchers have found that growers have realized substantial economic benefits with the adoption of herbicide-tolerant canola. A survey of canola growers in western Canada revealed that herbicide-tolerant technology generated from $1.063 – 1.192 billion in annual direct and indirect benefits over the 2005-2007 period partly attributed to lower input costs and partly attributed to better weed control (Smyth et al., 2010).

I.5. Alternatives

The decision-making process of deregulation is governed by 7 CFR § 340.6 (d)(3)(i) which states that APHIS may approve the petition in whole or in part, resulting in three possible outcomes from Monsanto's petition, described below.

[38] The USDA FAS reports do not distinguish canola but report all rapeseed.

I.5.1. Alternatives Studied in Detail

I.5.1.1. Deregulation in Whole Alternative

Under the "deregulation in whole" alternative, MON 88302 would no longer be a regulated article under 7 CFR Part 340 and would be widely available for planting without prior authorization in a permit or notification. With MON 88302, growers would have increased flexibility for glyphosate application on their canola crops, the option of applying glyphosate later in the growing season and the option to apply at higher rates.

I.5.1.2. No Action Alternative

Under the "no action" alternative, MON 88302 would remain a regulated article under 7 CFR Part 340. MON 88302 could be grown under USDA notification or permit and confined release conditions. However, currently deregulated herbicide-tolerant canola events including glyphosate-tolerant RT73 would continue to be available and would be expected to be widely grown.

I.5.2. Alternatives Considered and Dismissed

I.5.2.1. Approval in Part

The "approval in part" alternative is dependent upon a finding of potential plant pest risks for MON 88302 in certain geographies or under certain conditions. APHIS should impose conditions upon the cultivation or use of MON 88302 only in specific geographies or with specific conditions to mitigate any such identified plant pest risks. MON 88302 has been thoroughly characterized and the extensive information presented in Petition Sections I through IX demonstrates that MON 88302 does not present a plant pest risk in any of the geographies or under any conditions where MON 88302 may be grown. Therefore, there is no basis for imposing geographic or other conditions on MON 88302. Monsanto has requested an unconditional determination that MON 88302 poses no plant pest risk, and is therefore not subject to regulation under the Plant Protection Act.

I.6. Environmental Consequences

I.6.1. Commercial Canola Production and Use

No Action Alternative: Under the No Action Alternative, MON 88302 and its progeny would continue to be regulated articles under the regulations at 7 CFR part 340. Under this alternative planted canola acres in the U.S. may continue to increase in response to increases in consumer demand for canola oil. Based on recent trends, if increases in canola acreage occur, they will likely result from continued additions to wheat rotations (i.e., canola would be introduced as a rotation crop with wheat). General locations of production and uses of canola would be expected to continue as they are now. Herbicide-tolerant canola varieties would likely continue to be used on the vast majority of canola acres.

Deregulation in Whole Alternative: Biotechnology improved crops are subject to regulation in many countries. In order that canola seed harvested in the U.S. may be freely traded, Monsanto

will seek regulatory approval for MON 88302 and its combinations with other biotechnology-derived traits, where required, in all key canola import countries with a functioning regulatory system to support the flow of international trade (Petition Section VIII.K.). Monsanto adheres to the BIO Product Launch Policy[39] including: 1) conducting a market and trade assessment, 2) securing regulatory approvals in key export countries prior to full commercial launch, 3) following generally accepted best seed management practices to prevent unintended low level presence of the event in seed, 4) providing reliable detection methods to growers, processors and buyers prior to commercialization, and 5) communicating to stakeholders the company's product launch stewardship policies. These actions protect against adverse impacts to trade of canola due to the introduction of new biotechnology-derived canola.

Under deregulation in whole the same potential increases in canola acres planted may be expected for the same reason as for the no action alternative. The majority of commercial canola grown in the U.S. is already glyphosate-tolerant, and company stewardship policies described above ensure there will be no disruption of trade from the introduction of new biotechnology-derived products. The difference between the no action and the deregulation in whole alternative is expected to be the gradual replacement of the current Roundup Ready canola RT73 event with MON 88302. As described in Petition Sections VI. and VII., MON 88302 is compositionally and phenotypically equivalent to conventional canola. MON 88302 allows for a wider period of glyphosate application than RT73 at rates comparable to those already used for maize and soybean. The change in the biotechnology-derived trait, and the resulting allowable increase in glyphosate application, is not expected to have any impact on canola production and use. Therefore, the no action alternative and the approval in whole alternative would not differ in their impact to commercial canola production.

I.6.2. Seed Production

No Action Alternative: Under the No Action Alternative, MON 88302 and its progeny would continue to be regulated articles under the regulations at 7 CFR part 340. Under this alternative, seed production practices and geographies where canola seed is produced are expected to continue as described in Appendix Section I.4.2. The widespread use of herbicide-tolerant canola would likely continue, and canola seed production practices associated with herbicide-tolerant varieties is not expected to change.

Deregulation in Whole Alternative: Certified seed production is a carefully managed process (Petition Section VIII.B.) for maintaining high quality seed stocks, an essential basis for U.S. agriculture. Seed producers have learned to account for and manage pollen flow both within a seed production field and between nearby fields. For decades the canola seed industry has created and adopted systems to maintain and preserve the purity of canola seed developed for commodity and specialty uses. The Association of Seed Certifying Agencies (AOSCA) is dedicated to assisting companies in the production, identification, distribution and promotion of certified classes of seed. AOSCA establishes minimum standards for quality and identity. Its

[39] BIO's Product Launch guidelines can be found at: http://www.excellencethroughstewardship.org/facts/documents/Guide%20for%20Product%20Launch%20Stewardship.pdf.

goal is to standardize certification regulations and procedures internationally so companies compete with one set of standards. The association cooperates with the Organization for Economic Cooperation and Development (OECD) and other international organizations to develop standards, regulations, procedures, and policies to expedite movement of seed and encourage international commerce in improved seed products.

The difference between the no action and the deregulation in whole alternative is expected to be the gradual replacement of the current Roundup Ready canola RT73 event with MON 88302. It is anticipated that seed containing MON 88302 will be produced and marketed in accordance with OECD and AOSCA standards and the U.S. Federal Seed Act, and will have no adverse impact on current canola seed production practices or the ability of breeders and seed producers to meet these standards. The only change to seed production practices that may result from the deregulation of MON 88302 would be a potential change in weed management in seed production operations relative to practices used to produce existing glyphosate-tolerant varieties. Impacts on weed management from the introduction of MON 88302 are discussed in Appendix Section I.6.5. The change in the biotechnology-derived trait, and the potential changes in weed management, is not expected to have any impact on canola seed production. Therefore, the no action alternative and the approval in whole alternative would not differ in their impact to canola seed production.

I.6.3. Specialty Canola Production

No Action Alternative: Under the No Action Alternative, MON 88302 and its progeny would continue to be regulated articles under the regulations at 7 CFR part 340. Under this alternative, specialty canola production and other *Brassica* production, including *Brassica* vegetable production, is expected to continue as described in Appendix Sections I.4.3., I.4.4. and I.4.5. Specialty canola growers would continue to manage their production fields to avoid excluded methods including drift from pesticides

Additional new types of specialty canola may be introduced in the future. The very small acreages of organic canola production are likely to continue. Herbicide-tolerant canola will continue to be widely grown, and practices utilized to assure identity preservation of specialty canola would continue to be implemented.

Deregulation in Whole: Production systems designed prior to the introduction of MON 88302 or even prior to the introduction of biotechnology-derived canola have allowed for production of canola to meet varied customer demands. For example, organic canola producers use production practices designed to specifically avoid the presence of canola products that use herbicides or other pesticide treatments, as well as biotechnology-derived canola. These well established practices to avoid "excluded methods" will continue with the introduction of MON 88302. Currently, organic canola production occurs on a very small scale in the U.S., in areas removed from the major canola production areas. The change in the biotechnology-derived trait to MON 88302 will have no impact on organic canola production practices relative to the no action alternative

The primary *Brassica* vegetable crops in the U.S. are *B. napus* (rutabaga and Siberian kale), *B. oleracea* (cabbage, broccoli, cauliflower, Brussels sprouts, collard, kale, kohlrabi), *B. juncea*

(red giant mustard, sawtooth mustard, and others) and *B. rapa* crops (Chinese cabbage, pak choi, choi sum, turnip, mizuna, mibuna, tat soi and others). *Brassica* vegetables grown in the U.S. are harvested in the vegetative stage thus minimizing the potential for cross pollination with canola. Additionally, *Brassica* vegetable species are grown primarily in states such as California, Arizona, New York, Florida, Georgia, North Carolina, Wisconsin, Texas and Michigan where canola production is minimal. In states such as Washington and Oregon where *Brassica* vegetable crops and canola may both be grown, all *Brassica* seed crops are grown only through participation in a USDA extension pinning process that maps production sites and ensures adherence to appropriate isolation distances.

The difference between the no action and the deregulation in whole alternative is expected to be the gradual replacement of the current Roundup Ready canola RT73 event with MON 88302. The adoption of biotechnology-derived trait MON 88302 will have no impact on the current processes employed for planting and identity preservation in specialty canola, including organic canola or canola with modified oil composition or *Brassica* vegetables. Therefore, the no action alternative and the approval in whole alternative would not differ in their impact to specialty canola or *Brassica* vegetable production.

I.6.4. Agronomic Practices – Tillage, Irrigation, Crop Rotations, Insect and Disease Management

No Action Alternative: Under the No Action Alternative, MON 88302 and its progeny would continue to be regulated articles under the regulations at 7 CFR part 340 and would not be widely grown. Under this alternative, the agronomic practices for tillage, irrigation, crop rotation, insect pest management and disease management are expected to remain the same as those described in Appendix Section I.4.7.

Deregulation in Whole: MON 88302 has been shown to be no different from conventional canola in its agronomic and ecological characteristics (Petition Sections VII, VIII and IX), and has the same levels of resistance to insects and diseases as conventional canola. Therefore, other than applications of glyphosate for weed control, at rates comparable to those currently used for maize and soybean and a wider application window, there are no anticipated changes to current agricultural practices due to the introduction of MON 88302

The difference between the no action and the deregulation in whole alternative is expected to be the gradual replacement of the current Roundup Ready canola RT73 event with MON 88302. The adoption of biotechnology-derived trait MON 88302 will have no impact on the current agronomic practices of tillage, irrigation, crop rotations and insect and disease management. Therefore, the no action alternative and the approval in whole alternative would not differ significantly in their impact to canola agronomic practices.

I.6.5. Agronomic Practices - Weed Management

No Action Alternative: Under the No Action Alternative, MON 88302 and its progeny would continue to be regulated articles and would not be widely grown. Under this alternative, weed management in canola is expected to continue as described in Appendix Section I.4.7.9. Under the no action alternative, growers will likely continue to use the current Roundup Ready canola

RT73 and associated glyphosate use on approximately the same percentage of acres as currently. Other weed control practices would continue to be utilized at current rates. Based on U.S. EPA approved labels for Roundup agricultural herbicides, users of Roundup Ready canola RT73 can apply a maximum of 0.78 pounds acid equivalent (a.e.) per acre of glyphosate, from emergence to the 6-leaf stage. Growers can apply glyphosate once at a rate of up to 0.56 pound a.e. per acre or twice, at a rate of up to 0.39 pounds a.e. per acre. Weeds such as dandelion and Canada thistle would continue to be problematic in canola fields.

Deregulation in Whole Alternative: The difference between the no action and the deregulation in whole alternative is expected to be the gradual replacement of the current Roundup Ready canola RT73 event with MON 88302. As described in Petition Section VII. MON 88302 is phenotypically equivalent to conventional canola. Therefore, other than changes in herbicide use, weed management practices would not change. MON 88302 gives the grower additional options for glyphosate use. Monsanto has submitted amended labeling to the U.S. EPA that proposes to modify the current use pattern of glyphosate in canola based on MON 88302. If approved, MON 88302, growers will have the option to apply up to 1.55 pounds a.e. per acre of glyphosate from emergence to first flower. If the full 1.55 pounds a.e. per acre is applied at once, it must be done before the 6-leaf stage. Alternatively the grower may apply glyphosate twice, at rates up to 0.77 pounds a.e. per acre, up to first flower. These rates are similar to those currently allowed for soybeans (2.0 pounds a.e. per acre of glyphosate) and maize (2.0 pounds a.e. per acre of glyphosate), and based on weed control results in soybeans and maize at these glyphosate rates, it is expected that control of problematic weeds such as dandelion and Canada thistle would be improved. In addition, the increased window of glyphosate application will provide growers with greater flexibility in when they can apply glyphosate.

With the deregulation of MON 88302 glyphosate could be used for weed control in seed production acres which may result in a decrease in the application of some currently used herbicides. However Monsanto recommends, for weed control in hybrid seed production acres, and for all canola production acres that glyphosate be used in conjunction with pre-emergent herbicides to achieve the best possible control and mitigate concerns over the development of weed resistance (discussed in Appendix J). Additionally 3 to 4 year crop rotation practices are not expected to change as they are in place to minimize fungal disease cycles. These rotation practices also minimize the potential for the development of glyphosate resistant weeds.

Given the above, the adoption of biotechnology-derived trait MON 88302 will have no impact on the current weed management practices other than higher rates of glyphosate may be applied over a wider window. Therefore, the no action alternative and the approval in whole alternative would not differ significantly in their impact to canola weed management practices.

I.6.6. Volunteers

No Action Alternative: Under the No Action Alternative, MON 88302 and its progeny would continue to be regulated articles and would not be widely grown. Under this alternative, growers are expected to continue to use the management practices for control of canola volunteers as described in Appendix Section I.4.7.10.

Deregulation in Whole: The difference between the no action and the deregulation in whole alternative is expected to be the gradual replacement of the current Roundup Ready canola RT73 event with MON 88302. As described in Petition Section VII. , MON 88302 is equivalent to conventional canola in terms of seed germination, dormancy and emergence. In addition no differences were detected between MON 88302 and the conventional control for early stand count, seedling vigor, pod shattering, seed quality, yield and final stand count. Based on the assessed characteristics, the results of this study demonstrate that there were no unexpected changes indicative of increased plant pest potential or adverse environmental impact of MON 88302 compared to conventional canola. Therefore, control of volunteer canola is not expected to change from that described in Appendix Section I.4.7.10. In addition to using integrated weed management and cultural practices, growers may control volunteer MON 88302 canola with herbicides other than glyphosate and cultural practices just as they now control Roundup Ready canola RT73 volunteer canola with herbicides other than glyphosate and cultural practices.

I.6.7. Weed Resistance

No Action Alternative: Under the No Action Alternative, MON 88302 and its progeny would continue to be regulated articles and would not be widely grown. For the reasons described in Appendix Section I.4.7.11. and Appendix J, weeds that are glyphosate resistant are not currently an issue in canola production. With appropriate integrated weed management practices, this condition would be expected to continue. Under the no action alternative, growers are expected to continue to use current weed management practices.

Deregulation in Whole: The difference between the no action and the deregulation in whole alternative is expected to be the gradual replacement of the current Roundup Ready canola RT73 event with MON 88302. Canola, including glyphosate-tolerant canola, is produced in a crop rotation system that has relatively low susceptibility for development of weeds with herbicide resistance. As discussed in Appendix Section I.4.7.13, canola is typically grown in a three- to four-year rotation with small grains (wheat, barley and oats) that use herbicides with a different mode of action than glyphosate. The change from RT73 to MON 88302 will not affect these agronomic practices. Thus, the higher rate and wider period of glyphosate application that can be used with MON 88302 is not expected to have an impact on development of herbicide resistance in weeds.

I.6.8. Raw and Processed Agricultural Commodities

No Action Alternative: Under the No Action Alternative, MON 88302 and its progeny would continue to be regulated articles and would not be widely grown. Under this alternative the raw and processed agricultural commodities derived from canola are expected to be the same as described in Appendix Section I.4.8.

Deregulation in Whole: Biotechnology-derived canola products like MON 88302 undergo a voluntary food and feed consultation process with the U.S. FDA prior to release on the market. Monsanto has already initiated this process and will complete the consultation prior to a commercial introduction of MON 88302.

The difference between the no action and the deregulation in whole alternative is expected to be the gradual replacement of the current Roundup Ready canola RT73 event with MON 88302. Compositional assessments (Petition Section VI) conducted on MON 88302 seed support a conclusion that the composition of the seed is equivalent to that of the conventional control. The genetic modification in MON 88302, has no impact on the composition, and therefore on the food and feed safety or nutritional quality of this product compared to conventional canola. Residue tolerances for glyphosate in canola will not change.

Based on this information it is unlikely that the deregulation of MON 88302 would cause a significant impact on either raw or processed canola commodities. Therefore, the no action alternative and the approval in whole alternative would not differ significantly in their impact to canola weed management practices.

I.6.9. Potential for Cross Pollination and Gene Flow to Sexually Compatible Species

No Action Alternative: Under the No Action Alternative, MON 88302 and its progeny would continue to be regulated articles and would not be widely grown. As such, any plantings of MON 88302 would take place under APHIS notification or permit under conditions that minimize the potential for persistence of the regulated article in the environment. The potential for canola to cross pollinate in canola crops, canola seed production, other *Brassica* crop production, other *Brassica* seed production and the potential for cross pollination to native species and weeds, is discussed in Appendix Section I.4.9. As discussed in that section, there are reports of unassisted (e.g. without human intervention) hybridization under field conditions with *B. napus* as the pollen donor with six other species including *B. rapa*, *B. juncea*, *B. oleracea*, *H. incana*, *R. raphanistrum* and *S. arvensis* (see Petition, Table IX-3). In all cases the resulting hybrids had decreased environmental fitness evidenced by a variety of characteristics including decreased pollen viability, seed production and seedling survival, when compared to parental varieties.

As a result of state and federal regulatory requirements, seed certification requirements, seed grower agreements, geography, and/or biological constraints provide barriers to cross pollination between *B. napus* and other closely related species during seed production. Thus cross pollination during seed production is minimal to non-existent. Under the no action alternative growers are expected to continue to use biotechnology-derived, herbicide-tolerant canola having the same potential for cross pollination with sexually compatible species as conventional canola.

Deregulation in Whole: The difference between the no action and the deregulation in whole alternative is expected to be the gradual replacement of the current Roundup Ready canola RT73 event with MON 88302. As described in Petition Section VII. , MON 88302 is phenotypically equivalent to conventional canola and is not expected to have a greater potential for cross pollination with related species than conventional canola. Therefore, the no action alternative and the approval in whole alternative would not differ significantly in their impact to cross pollination in canola.

I.6.10. Ruderal Canola

No Action Alternative: Under the No Action Alternative, MON 88302 and its progeny would continue to be regulated articles and would not be widely grown. Most ruderal canola results from seed loss during transportation and is found growing at the edges of roadways and along alternative transportation routes. Multiple studies of roadside or ruderal canola have found that canola populations generally persist only for a year or two, and are usually found within a few feet of a roadway in disturbed soil not yet supplanted by grasses and other more aggressive plants. The current status of ruderal canola, is not expected to change under the no action alternative, and is discussed in detail in Appendix Section I.4.10.

Deregulation in Whole: The difference between the no action and the deregulation in whole alternative is expected to be the gradual replacement of the current Roundup Ready canola RT73 event with MON 88302. In studies biotechnology-derived canola populations have not been found to be more invasive or more persistent than conventional canola populations, and biotechnology-derived canola seed has not been demonstrated to persist longer than conventional canola seed. The introduction of MON 88302 is not expected to impact canola survival or control as compared to conventional canola. Thus, the no action alternative and the approval in whole alternative would not differ significantly in their impact on ruderal canola.

I.6.11. Public Health and Worker Safety

No Action Alternative: Under the No Action Alternative, MON 88302 and its progeny would continue to be regulated articles and would not be widely grown. Under this alternative, potential affects to public health relating to the production of canola are expected to remain unchanged and similar to those described in Appendix Section I.4.11. Canola is expected to continue to be grown at approximately the same scale and in the same geographic areas. Herbicides, including glyphosate, will continue to be used at current rates as authorized by the U.S. EPA with reasonable certainty of no harm.

Deregulation in Whole Alternative: Prior to the introduction of a biotechnology-derived crop product to the marketplace, Monsanto conducts tests to assure that the product is as safe as its conventional counterpart under the intended use conditions. Biotechnology-derived crops for food and feed use undergo a voluntary consultation process with the U.S. FDA prior to release into the market. Monsanto will complete the U.S. FDA consultation process prior to the introduction of MON 88302.

Under the Federal Food, Drug, and Cosmetic Act (FFDCA), pesticide residues in or on raw agricultural commodities or processed foods are allowed only after a tolerance or exemption from tolerance has been established. Residue tolerances and exemptions for pesticides are established by the U.S. EPA under the FFDCA. The U.S. FDA enforces the tolerances set by the U.S. EPA. The U.S. EPA also reviews the proposed use pattern for all herbicides and prior to approval and placement on herbicide labels determines that no unreasonable risk exists for the environment.

The difference between the no action and the deregulation in whole alternative is expected to be the gradual replacement of the current Roundup Ready canola RT73 event with MON 88302. As

summarized in Petition Section V.F, the CP4 EPSPS protein expressed in MON 88302, as in other Roundup Ready crops, does not pose a significant health risk. MON 83302 expresses the CP4 EPSPS protein throughout the plant conferring tolerance to glyphosate, which is the active ingredient in the Roundup family of agricultural herbicides. It is structurally homologous to EPSPS proteins that are part of the amino acid synthesis pathway of all plants (Devine, et al., 1993). The safety of any protein(s) newly introduced into a biotechnology-derived crop needs to be assessed (Delaney, et al., 2008; ILSI, 2004). The safety of CP4 EPSPS protein present in biotechnology-derived crops has been extensively evaluated (Harrison et al., 1996; ILSI-CERA, 2010). The U.S. EPA has also reviewed the safety of the CP4 EPSPS protein and has established a tolerance exemption for the protein and the genetic material necessary for its production in or on all raw agricultural commodities (40 CFR § 174.523). This exemption was based on a safety assessment that included rapid digestion in simulated gastric fluids, lack of homology to known toxins and allergens, and lack of toxicity in an acute oral mouse gavage study. A history of safe use is supported by the lack of any documented reports of adverse effects since the introduction of other Roundup Ready crops in 1996.

Roundup Ready canola RT73 is planted on greater than 50% of U.S. canola acreage (USDA-NASS, 2008). Consequently glyphosate is the most widely used herbicide in canola. The toxicology of glyphosate has been extensively reviewed. A summary of food and feed tolerances, regulatory approvals with associated dietary exposure assessments and recent chronic and short-term aggregate risk assessments for glyphosate has been conducted by the U.S. EPA.

In regards to canola nutritional and compositional aspects, as described in Petition Section VI, extensive compositional analyses of canola seed were conducted on samples from replicated, multi-site field trials to compare the composition of MON 88302 to a conventional canola control and to commercially available canola varieties. The compositional analyses confirmed that for the components analyzed in MON 88302 seed, that MON 88302 is compositionally equivalent to conventional canola that has a history of safe use.

Workers may be exposed to higher levels of glyphosate with MON 88302 because of the proposed higher glyphosate application rates relative to currently registered use patterns on canola. However, recall that the proposed rates of glyphosate applications are comparable to rates already used in maize and soybean (2 pounds a.e. per acre of glyphosate in-crop). The affected environment for pesticide use and human health is discussed in Appendix Section I.4.11.2. That section describes the U.S. EPA's conclusions and the conclusions of other studies regarding worker exposure, and incidental exposure to non-workers who may be in the vicinity where glyphosate is being applied. Currently the U.S. EPA is undergoing a separate analysis related to the glyphosate use pattern requested for use with MON 88302.

I.6.12. Animal Health

No Action Alternative: Under the No Action Alternative, MON 88302 and its progeny would continue to be regulated articles and would not be widely grown. Under this alternative, the use of canola and its processed fractions as a feed source is expected to continue as described in Appendix Section I.4.12. Under the no action alternative, farmers will likely continue to plant Roundup Ready canola RT73 as a feed source at approximately the same rate as currently,

continue to apply glyphosate at label rates to control weeds and the harvested seed will continue to be used as a feed source.

Deregulation in Whole Alternative: The difference between the no action and the deregulation in whole alternative is expected to be the gradual replacement of the current Roundup Ready canola RT73 event with MON 88302. As described in Petition Section V, the CP4 EPSPS protein expressed in MON 88302, as in other Roundup Ready crops, does not pose a significant animal health risk. As discussed previously, there is no meaningful risk to animal health from dietary exposure to CP4 EPSPS produced in MON 88302. There are no toxic properties associated with the CP4 EPSPS protein (Harrison, et al., 1996). The estimated animal dietary feed mean intake of CP4 EPSPS protein from MON 88302 ranges from 0.23 to 0.80 mg/kg body weight per day depending on the animal species. No adverse effects were observed at very high CP4 EPSPS consumption levels (572 mg/kg body weight) (Harrison et al., 1996). Furthermore, the composition of the seed produced by MON 88302 is unchanged compared to conventional canola. This information indicates that there would be no negative effects to animals consuming fractions derived from MON 88302.

The food and feed safety assessment Monsanto submitted to U.S. FDA (discussed in Appendix Section I.3.1.) concluded that feed derived from MON 88302 is as safe and nutritious as feed derived from conventional canola.

I.6.13. Animal, Plant and Microbial Communities Including Threatened and Endangered Species

I.6.13.1. Animal Communities

No Action Alternative: Under the No Action Alternative, MON 88302 and its progeny would continue to be regulated articles and would not be widely grown. Under this alternative, the effect of canola on animal communities is expected to continue as described in Appendix Section I.4.13. Canola is expected to continue to be grown in approximately the same amounts and areas and glyphosate would continue to be used on canola acres.

Deregulation in Whole Alternative: The difference between the no action and the deregulation in whole alternative is expected to be the gradual replacement of the current Roundup Ready canola RT73 event with MON 88302. As summarized in Petition Section V.F, the CP4 EPSPS protein expressed in MON 88302, as in other Roundup Ready crops, does not pose a significant health risk to animals. The safety of CP4 EPSPS protein present in biotechnology-derived crops has been extensively evaluated (Harrison et al., 1996) and reviewed by the U.S. EPA which established a tolerance exemption for the protein and the genetic material necessary for its production in or on all raw agricultural commodities (40 CFR § 174.523). A history of safe use is supported by the lack of any documented reports of adverse effects since the introduction of the first Roundup Ready crop in 1996.

The composition of the harvested seed produced by canola containing MON 88302 is comparable to conventional canola (Petition Section VI). Furthermore, it is unlikely that canola fields available for foraging would contain high amounts canola seed after harvest. This information indicates that there would be no negative effects to mammals that forage on

MON 88302. Similarly, it is expected that there would be no impact to birds or other animals, including migratory birds and animals, that may consume MON 88302 seed.

During field trials no changes in insect feeding damage were observed (Petition Section VII.) indicating similar insect susceptibility for MON 88302 compared to conventional canola. Additionally, in a quantitative assessment of pest and beneficial arthropod abundance, no statistically significant differences were detected between MON 88302 and the conventional control. As MON 88302-containing canola exhibits no toxic effects on animals or pollinators of other plants in or around fields cultivated with MON 88302, it is unlikely insects and animals will be significantly affected.

MON 88302 gives the grower additional options for glyphosate use. Growers will have the option to apply up to 1.55 pounds a.e. per acre of glyphosate from emergence to first flower. This maximum rate is similar to rates currently allowed for soybean and maize and is less than one third the rate (5.0625 pounds a.e. per acre) used in the U.S. EPA's ecological assessment for glyphosate (Appendix Section I.4.13.). In its analysis, the U.S. EPA used the 5.0625 pounds a.e.per acre rate to calculate the estimated environmental concentrations (EECs) for aquatic plants and animals. Based on this assessment, it was concluded that effects to birds, mammals, fish and invertebrates are minimal (U.S. EPA, 1993).

Based on the analyses presented Appendix Section I.4.13. and the maximum application rate of glyphosate at 1.55 pounds a.e. per acre, glyphosate use associated with MON 88302 is not expected to have any impacts on animal communities in or adjacent to canola fields. Thus, the no action alternative and the approval in whole alternative would not differ significantly in their impact on animal communities.

I.6.13.2. Plant Communities

No Action Alternative: Under the No Action Alternative, MON 88302 and its progeny would continue to be regulated articles and would not be widely grown. Under the no action alternative, the effect of canola on plant communities is expected to continue as described in Appendix Section I.4.13. Canola and glyphosate-tolerant canola are expected to continue to be grown in approximately the same amounts and areas and glyphosate would continue to be used on canola acres.

Deregulation in Whole Alternative: The potential for MON 88302 to impact nearby vegetation is related to its weediness potential, which could result in the uncontrolled spread into surrounding environments, as well as its ability to interbreed with nearby sexually compatible plants. In addition, off-target movement from spray or vapor drift occurs with all herbicide applications, and effects on non-target plants do occasionally occur as a result of their use. The degree of injury to non-target plants that may occur from off-target movement is dependent on the sensitivity of the plant to the herbicide; however these impacts can be minimized through good management practices such as decreasing spray pressure, lowering boom height, increasing nozzle size, avoiding making applications during high winds, etc. (Jordan, et al., 2009; University of Illinois, 2010).

The potential for canola to outcross in canola crops, canola seed production, other *Brassica* crop production, other *Brassica* seed production and the potential for cross pollination to native species and weeds, is discussed in Appendix Section I.4.9. As discussed in that section, there are reports of unassisted (e.g. without human intervention) hybridization under field conditions with *B. napus* as the pollen donor with six other species including *B. rapa*, *B. juncea*, *B. oleracea*, *H. incana*, *R. raphanistrum* and *S. arvensis* (see Petition Table IX-3). In all cases the resulting hybrids had decreased environmental fitness evidenced by a variety of characteristics including decreased pollen viability, seed production and seedling survival, when compared to parental varieties.

There is also the potential for ruderal canola populations to form at the edges of roadways and fields where canola is grown. Multiple studies of ruderal canola have found that canola populations generally persist only for a year or two, and are usually found in disturbed soil not yet supplanted by grasses and other more aggressive plants. In studies biotechnology-derived canola populations have not been found to be more invasive or more persistent than conventional canola populations, and biotechnology-derived canola seed has not been demonstrated to persist longer than conventional canola seed.

The difference between the no action and the deregulation in whole alternative is expected to be the gradual replacement of the current Roundup Ready canola RT73 event with MON 88302. MON 88302 does not exhibit characteristics associated with weedy growth and will not compete with plants found outside of agricultural production. Thus the introduction of MON 88302 does not provide increased potential for cross pollination with closely related species or increased potential for the development of ruderal populations over conventional canola or the current Roundup Ready canola RT73.

As discussed in Appendix Section I.4.11.2, glyphosate is a non-selective herbicide with post-emergence activity on essentially all annual and perennial plants. As such, exposure to glyphosate could affect aquatic and terrestrial nontarget plants (U.S. EPA, 1993). In general, plants may potentially be affected from applications of herbicides as a result of spray drift or runoff. Glyphosate, however, binds strongly to agricultural soils and has low potential to move offsite dissolved in water (U.S. EPA, 1993). Moreover, glyphosate is not taken up from agricultural soils by plants. Therefore, any potential glyphosate effects to nontarget plants are only due to exposure via spray drift. As described in Appendix Section I.4.13., using methodology from a recent U.S. EPA effects determination, it can be determined that terrestrial plants that are not threatened or endangered are unlikely to be significantly impacted by glyphosate applications to MON 88302. In addition, glyphosate labels include specific risk management measures to manage spray drift, including mandatory requirements for aerial applications.

Therefore, the no action alternative and the approval in whole alternative would not differ significantly in their impact on plant communities.

I.6.13.3. Soil Microorganisms

No Action Alternative: Under the No Action Alternative, MON 88302 and its progeny would continue to be regulated articles and would not be widely grown. Under this alternative the effect of canola on soil microorganisms is expected to continue as described in Appendix Section I.4.13. Canola and glyphosate-tolerant canola is expected to continue to be grown in approximately the same amounts and areas and glyphosate would continue to be used on canola acres.

Deregulation in Whole Alternative: The difference between the no action and the deregulation in whole alternative is expected to be the gradual replacement of the current Roundup Ready canola RT73 event with MON 88302. No adverse effects on soil microorganisms are associated with MON 88302 nor do the characteristics of the CP4 EPSPS protein pose any concern to soil microorganisms (ILSI-CERA, 2010). Therefore, MON 88302 is not expected to have any impacts on soil microorganisms.

The effects of glyphosate on soil microorganisms have been extensively investigated (Giesy et al., 2000; Sullivan and Sullivan, 2000). Long-term studies following repeated applications of glyphosate in the field for six (Olson and Lindwall, 1991) or over ten years (Biederbeck, et al., 1997; Hart and Brookes, 1996) have shown no detectable adverse effects on soil microbes. Investigations by Haney (2002; 2000) related to the increased use of glyphosate-tolerant crops demonstrated that glyphosate was degraded over time by soil microorganisms without adversely impacting soil microbial communities. Based on this body of evidence, no significant impacts on soil microorganisms are anticipated from the deregulation of MON 88302 and subsequent applications of glyphosate.

I.6.13.4. Threatened and Endangered Species

No Action Alternative: Under the No Action Alternative, MON 88302 and its progeny would continue to be regulated articles and would not be widely grown. Under this alternative, the potential impact of canola on threatened or endangered (TE) species as defined by the U.S. FWS is expected to remain the same. Canola itself does not impact TE species. Canola is not sexually compatible with any listed TE plant or plant proposed for listing, or a host of any TE species because there are no listed species or species proposed for listing in the genus *Brassica* or that would use *Brassica* species as a host. As summarized in Petition Section V.F, the CP4 EPSPS protein expressed in RT73, as in other Roundup Ready crops has no effect on any TE species.

No reports of any impacts to TE species from the use of glyphosate applied to canola have been found, and none would be expected, based on the application rates, as discussed in Section 1.4.13. Under the no action alternative, growers are expected to continue to use RT73 in approximately the same amounts and areas, with potentially some increase in acreage.

Deregulation in Whole Alternative: The difference between the no action and the deregulation in whole alternative is expected to be the gradual replacement of the current Roundup Ready canola RT73 event with MON 88302.

As with the no action alternative, canola itself is not sexually compatible with any TE plant species; therefore there is no potential for a direct effect of MON 88302 on TE plants.

As with the no action alternative, MON 88302 expresses the CP4 EPSPS protein. As summarized in Petition Section V.F, the CP4 EPSPS protein does not pose a significant allergenic risk to humans or animals and the donor organism for the CP4 EPSPS coding sequence, *Agrobacterium* sp. strain CP4, is ubiquitous in the environment and is not commonly known for human or animal pathogenicity or allergenicity. The CP4 EPSPS protein lacks structural similarity to allergens, toxins or other proteins known to have adverse effects on mammals. The CP4 EPSPS protein is rapidly digested in simulated digestive fluids and demonstrates no oral toxicity in mice at the level tested. Based on the above information, the consumption of the CP4 EPSPS protein from MON 88302 or its progeny are considered safe for humans and animals.

As the action agency for pesticide registrations, the U.S. EPA has the responsibility to conduct an assessment of effects of a registration action on threatened and endangered species (TES). The U.S. EPA Endangered Species Protection Program web site, http://www.epa.gov/espp/, describes the U.S. EPA assessment process for endangered species. Some of the elements of that process, generally taken from the web site, are summarized below.

When registering a pesticide or reassessing the potential ecological risks from use of a currently registered pesticide, the U.S. EPA evaluates extensive exposure and ecological effects data to determine how a pesticide will move through and break down in the environment. Risks to birds, fish, invertebrates, mammals and plants are routinely assessed and used in the U.S. EPA's determinations of whether a pesticide may be licensed for use in the U.S.

The U.S. EPA's core pesticide risk assessment and regulatory processes ensure that protections are in place for all populations of nontarget species, including TE species. These assessments provide the U.S. EPA with information needed to develop label use restrictions for the pesticide. These label restrictions carry the weight of law and are enforced by U.S. EPA and the states (Federal Insecticide, Fungicide, and Rodenticide Act 7 USC 136j (a)(2)(G) Unlawful acts). Because threatened and endangered species may need specific protection, the U.S. EPA has developed risk assessment procedures described in the *Overview of the Ecological Risk Assessment Process* (U.S. EPA, 2004) to determine whether individuals of a listed species have the potential to be harmed by a pesticide, and if so, what specific protections may be appropriate. The U.S. EPA's conclusion regarding the potential risks a pesticide may pose to a listed species and any designated critical habitat for the species, after conducting a thorough ecological risk assessment, results in an "effects determination" in accordance with Section 7 (a)(2) of the Endangered Species Act.

As a part of the U.S. EPA's threatened and endangered species effects assessment for the California red-legged frog (U.S. EPA 2008b), the U.S. EPA evaluated the effect of glyphosate use at rates up to 7.95 lbs a.e. glyphosate per acre on fish, amphibians, aquatic invertebrates, aquatic plants, birds, mammals, and terrestrial invertebrates. This assessment determined that at the maximum application rate for in-crop applications of glyphosate to MON 88302 (1.55 lbs a.e. glyphosate per acre) there would be no effects of glyphosate use on the following taxa of threatened and endangered species: fish, amphibians, birds, and mammals. The U.S. EPA assessment was uncertain of the effects on terrestrial invertebrates, citing the potential to affect small insects at all application rates and large insects at the higher application rates. The U.S. EPA considered these potential effects as part of their review process and label use restrictions

imposed under authority of FIFRA. To mitigate potential adverse effects to threatened and endangered species, the U.S. EPA has imposed specific label use restrictions for glyphosate use when applied with aerial equipment including "The product should only be applied when the potential for drift to adjacent sensitive areas (e.g., residential areas, bodies of water, known habitat for threatened or endangered species, non-target crops) is minimal (e.g., when wind is blowing away from the sensitive areas)."

In conclusion, there are legal precautions in place (U.S. EPA label use restrictions) and "best practice" guidance to reduce the possibility of exposure and adverse impacts to TE species from glyphosate application to MON 88302; the U.S. EPA has considered potential impacts to TE species as part of their registration and labeling process for glyphosate; and adherence to the U.S. EPA label use restrictions by the pesticide applicator will ensure that the use of glyphosate will not adversely affect threatened and endangered species or critical habitat. Based on these factors and the legal requirements for pesticide applicators to follow the U.S. EPA label use restrictions, the use of the U.S. EPA registered glyphosate for MON 88302 production will not adversely impact listed species or species proposed for listing and will not adversely impact designated critical habitat or habitat proposed for designation.

I.6.14. Physical Environment

I.6.14.1. Land Use

No Action Alternative: Under the No Action Alternative, MON 88302 and its progeny would continue to be regulated articles and would not be widely grown. Under this alternative, impacts of canola production on land use are expected to continue as described in Appendix Section I.4.14. Under the no action alternative, growers will likely continue to use Roundup Ready canola RT73 in the same areas and with the same weed control options, at approximately the same rate of glyphosate applications as currently.

Deregulation in Whole Alternative: The difference between the no action and the deregulation in whole alternative is expected to be the gradual replacement of the current Roundup Ready canola RT73 event with MON 88302.

Glyphosate-tolerant canola has been deregulated and grown in the U.S. since 1999. Roundup Ready canola RT73 currently occupies greater than 50% of total canola commercial acres. The acreage of glyphosate-tolerant canola increased rapidly following commercial introduction and has remained between 50 and 70% of planted canola acres since 2001. Fluctuations in total canola acreage before and after glyphosate-tolerant canola was commercialized (Figure I-3) indicates that factors unrelated to the availability of the glyphosate-tolerant trait play a larger role in acres planted than the availability of the glyphosate-tolerant trait.

Despite the rapid adoption of herbicide-tolerant canola products in the past decade, there has been no significant impact on total cropland acreage in the U.S. For example, from 2001 to 2010, the total annual commercial canola acres planted averaged between 0.8M to 1.5M acres (Petition Table VIII-1), while in the same time frame, the adoption rate for biotechnology-derived herbicide-tolerant canola in North Dakota increased from 0% to 95% (Zollinger et al., 2009). Agricultural land use, and consequently crop production is dictated by many factors, the

most significant of which are commodity prices. Accordingly, growers may increase acres dedicated to canola production to meet increased need, but they do so in response to commodity prices and market demand, not in response to availability or adoption of biotechnology-derived traits. Thus, the introduction of MON 88302 is not anticipated to facilitate production of canola in areas where it is not currently grown or have significant impact on total canola production acres.

I.6.14.2. Air Quality and Climate Change

No Action Alternative: Under the No Action Alternative, MON 88302 and its progeny would continue to be regulated articles and would not be widely grown. Potential impacts on air quality and climate change associated with canola production are not expected to change under the no action alternative (Appendix Section I.4.14.). Under the no action alternative, growers will continue to use Roundup Ready canola RT73 and may change tilling practices and no-till adoption rates or use different herbicide regimes, which could have very short-term impacts on air quality.

Deregulation in Whole Alternative: The difference between the no action and the deregulation in whole alternative is expected to be the gradual replacement of the current Roundup Ready canola RT73 event with MON 88302. Growers may use glyphosate application rates with MON 88302 that are higher than the current Roundup Ready canola RT73, but still comparable to the rates used on maize and soybean at 1.7% and 1.9% the amount of acres respectively. These increased application rates may result in temporary, very short-term increases in herbicide levels in the air. Therefore, the introduction of MON 88302 is not anticipated to result in significant changes in air quality or climate change.

I.6.14.3. Surface and Groundwater Quality

No Action Alternative: Under the No Action Alternative, MON 88302 and its progeny would continue to be regulated articles and would not be widely grown. Potential impacts on surface water and groundwater, which are not expected to change under the no action alternative, are discussed in detail in Appendix Section I.4.14. Under the no action alternative, growers will continue to use RT73 and may change tilling rates or use of different herbicides over time, which could have very short-term impacts on surface and groundwater.

Deregulation in Whole Alternative: Water quality could be impacted either directly by MON 88302 via plant material impacts on water resources, or indirectly via impacts from the use of glyphosate or tillage practices associated with the planting of MON 88302. Conservation tillage, a system that leaves 30% or more of the previous crop residue covering the soil when planting another crop has been increasingly employed in commercial canola acres, and helps minimize any impacts of canola production on water quality by reducing soil erosion.

In terms of potential direct impacts on water quality, the CP4 EPSPS protein contained in MON 88302 is a member of the larger family of EPSPS proteins that are ubiquitous in plants and microbes in the environment. The mode of action of this family of proteins is well known and the introduced CP4 EPSPS protein itself was derived from a common soil bacterium (*Agrobacterium sp*. strain CP4). The safety of CP4 EPSPS protein present in other glyphosate-

tolerant crops has been extensively evaluated, and the U.S. EPA has granted a tolerance exemption for CP4 EPSPS. A history of safe use of CP4 EPSPS is supported by the lack of any documented reports of adverse effects since the introduction of Roundup Ready crops. Under full deregulation of MON 88302, current grower practices related to canola production, including weed control and tillage practices would not be significantly altered, as greater than 50% of current canola acres already contain the same glyphosate tolerance trait as in MON 88302. Therefore, it is unlikely that the presence of CP4 EPSPS protein in MON 88302 will have a significant impact on water quality.

The difference between the no action and the deregulation in whole alternative is expected to be the gradual replacement of the current Roundup Ready canola RT73 event with MON 88302. MON 88302 gives the grower additional options for glyphosate use. Growers will have the option to apply up to 1.55 pounds a.e. per acre of glyphosate from emergence to first flower. These rates are similar to those currently allowed for soybeans and maize. Glyphosate has been thoroughly reviewed by the U.S. EPA and has been determined to "not pose unreasonable risks or adverse effects to humans or the environment" (U.S. EPA, 1993). Glyphosate is rapidly adsorbed and tightly complexed by soil particles and, even though it is highly water soluble, it does not leach into ground water in most soils. In intensely farmed areas, herbicides have often been found in surface waters due principally to rainfall runoff. With greater soil sorptivity glyphosate is found at lower concentrations than other herbicides such as atrazine and alachlor, and it has been shown to dissipate more rapidly than other herbicides in surface water (Carpenter, et al., 2002; Cerdeira and Duke, 2006). Therefore, it is unlikely that the use of glyphosate on MON 88302 will have a significant impact on water quality.

I.6.15. Socio-Economic Impacts

No Action Alternative: Under the No Action Alternative, MON 88302 and its progeny would continue to be regulated articles and would not be widely grown. Economic conditions associated with growing canola, which are not expected to change under the no action alternative, are discussed in Appendix Section I.4.15. Under the no action alternative, growers will likely continue to use Roundup Ready canola RT73 at approximately the same, or potentially a slightly increased rate as currently, and will continue to use current weed control practices.

We found no cases of organic canola growers who have been affected by the need for increased isolation distances because of the presence of RT73, but did find that one organic canola grower made the decision to switch from canola to other organic crops when biotechnology-derived canola was introduced in the U.S. Another organic grower considered canola, but chose a different oilseed crop because of concerns about disease and insect management.

Deregulation in Whole Alternative: Total global U.S. imports of canola oil continue to increase steadily from 0.5 million metric tons in 2000 (USDA-FAS, 2011a) to 1.2 million metric tons in 2010 (USDA-FAS, 2011b). Although canola production in the U.S. has increased dramatically since the 1980s, it has always been far short of demand (Figure I-2). The U.S. shortfall is made up with imports primarily from Canada.

Although canola has been able to benefit from the same price subsidies as other commodity crops, over a ten year period canola acres have not grown substantially (Petition Table VIII-1) even after the introduction of the current Roundup Ready canola RT73. The difference between the no action and the deregulation in whole alternative is expected to be the gradual replacement of the current Roundup Ready canola RT73 event with MON 88302. It is not expected that the introduction of MON 88302 will result in significantly increased acres, and any potential impacts to organic or specialty organic growers would be the same as for the no action alternative. Weed management practices would change slightly as growers would be able to apply higher rates of glyphosate for a greater period of time during canola's life cycle. Thus the costs of weed management may rise incrementally. However, researchers have found that growers have realized substantial economic benefits with the adoption of herbicide-tolerant canola. A survey of canola growers in western Canada revealed that herbicide-tolerant technology generated from $1.063 – 1.192 billion in annual direct and indirect benefits over the 2005-2007 period partly attributed to lower input costs and partly attributed to better weed control (Smyth et al., 2010). Therefore it is unlikely the deregulation of MON 88302 will have a significant negative impact on grower economics.

I.6.16. Cumulative Impacts

CEQ regulations define cumulative impacts as "the impact on the environment which results from the incremental impact of the action when added to other past, present, and reasonably foreseeable future actions regardless of what agency (federal or non-federal) or person undertakes such other action."[40] Thus, cumulative impacts are assessed only for those resources that are impacted by the proposed action. Cumulative impacts may result from individually minor, but collectively significant, actions taking place over time.

Cumulative impacts occur when the effects of an action are added to the effects of other actions occurring in a specific geographic area and timeframe. The cumulative impact analysis follows CEQ's guidance: Considering Cumulative Effects under the National Environmental Policy Act (CEQ, 1997). The steps associated with the analysis include:

- Specify the class of actions for which effects are to be analyzed.
- Designate the appropriate time and space domain in which the relevant actions occur.
- Identify and characterize the set of receptors to be assessed.
- Determine the magnitude of effects on the receptors and whether those effects are accumulating.

This section discusses the cumulative impacts that are associated with the deregulation in whole alternative, when combined with other recent past, present, and reasonably foreseeable future actions within the affected environment.

[40] 40 CFR 1508.7

Cumulative Impacts of Potential Increased Glyphosate Usage

No Action Alternative: Under the No Action Alternative, MON 88302 and its progeny would continue to be regulated articles and would not be widely grown. For MON 88302 the only impacts are those associated with the increased application rate of glyphosate. Under the no action alternative, weed management in canola is expected to continue as described in Appendix Section I.4.7.9. Growers will likely continue to use the current Roundup Ready canola RT73 and associated glyphosate use on approximately the same percentage of acres as currently. Other weed control practices would continue to be utilized at current rates. Based on U.S. EPA approved labels for Roundup agricultural herbicides, users of Roundup Ready canola RT73 can apply a maximum of 0.78 pounds acid equivalent (a.e.) per acre of glyphosate, from emergence to the 6-leaf stage. As shown in Table I-11 canola currently accounts for 0.7% of total glyphosate use and this would not be expected to change significantly.

Deregulation in Whole Alternative: The impact of the deregulation in whole alternative results from the option to use increased application rates for glyphosate. Users of RT73 can apply a maximum of 0.78 pounds a.e. per acre of glyphosate, from emergence to the 6-leaf stage. With MON 88302, growers will have the option to apply up to 1.55 pounds a.e. per acre of glyphosate from emergence to first flower. If the full 1.55 pounds a.e. per acre is applied at once, it must be done before the 6-leaf stage. Alternatively the grower may apply glyphosate twice, at rates up to 0.77 pounds a.e. per acre, up to first flower.

Temporal Effects: Cumulative impacts can be important for herbicides or pesticides that persist in the environment and thus can continue to accumulate with repeated applications. Glyphosate is not persistent and does not accumulate. In its re-registration decision for glyphosate (U.S. EPA 1993), the U.S. EPA reported a median half-life of 13.9 days for glyphosate, when applied at the maximum annual rate of 7.95 lb a.e./acre. Colder climates tended to have higher half-lives: the half-life for Minnesota, the closest state to North Dakota that was reported, was 28.7 days. Thus, if all glyphosate used stopped today, its effects, both positive and negative, would soon be negligible. Therefore, the temporal cumulative effects of increased glyphosate use are very limited.

Geographic Effects: Because glyphosate binds tightly to soils and drift affects a small area adjacent to a canola field, cumulative geographic effects are also very limited. In other words, the area of influence of a MON 88302 canola field in terms of glyphosate impacts is the field plus a small adjacent area that could potentially be impacted by drift. Cumulative effects would occur only when the area of influence for some other glyphosate application intersected with the area of influence for a MON 88302 canola field. Thus, the cumulative geographic effects are also very limited.

MON 88302 Use in Context: Table I-11 shows estimated 2010 glyphosate use in the U.S. As shown, canola currently accounts for 0.7% of the total use. Assuming the adoption of the maximum rate (1.55 pounds a.e. per acre, which is an increase from 0.78 pounds a.e. per acre) is used for MON 88302, canola would account for approximately 1.6% of the total glyphosate use in the U.S. In the context of overall U.S. glyphosate use, this is a negligible change.

Table I-11. Glyphosate Use on Roundup Ready Crops

RR Crops	lbs Glyphosate per acre[1]	RR Adoption[2]	Total Acres (million)[3]	RR Acres (million)	lbs Glyphosate (thousand)	% of Total
Maize	0.95	0.7	87.9	61.5	58454	25.1
Cotton	1.89	0.78	10.9	8.5	16069	6.9
Soybean	1.36	0.93	79	73.5	99919	42.9
Canola	1.125	0.9	1.5	1.4	1519	0.7

[1]Maize, cotton, soybean glyphosate rates from (Benbrook, 2009) canola rate from NDSU (2007b).
[2] USDA-NASS, 2010.
[3]Zollinger et al., 2009.

Attachment 1: Appendix I. *Brassica* and Related Vegetable Seed Production

Vegetable seed crops are a very small part of U.S. agricultural output and published data is not widely available. According to the 2007 Census of Agriculture, the total U.S. vegetable seed field acreage in 2007 was 45,309, less than one percent of the total harvested vegetable acreage of 4,682,588 (USDA NASS 2009). Based on the analysis below, it appears that nearly all the non-broccoli *Brassica* seed production occurs in Washington and Oregon and nearly all the broccoli seed production occurs in California and Arizona.

Washington and Oregon Production

The majority of the *Brassica* seed grown in the U.S. is produced in three areas of Washington and Oregon: in western Washington in the lower-lying areas around the Puget Sound between the Olympic Mountains and the Cascades; in the Columbia Basin of eastern Washington; and in Oregon's Willamette Valley, located between the Coastal Range and the Cascades.

The most comprehensive information is available for Washington State. Based on the discussion below, the total estimated Washington acreage of *Brassica* and related vegetable seed production is approximately 4,500 acres, of which approximately 43% (1,935 acres) is planted in *Brassica* species, with the rest being planted with crops such as arugula, daikon and radish, members of the family *Brassicaceae* but not members of the *Brassica* genus. Attachment Table 1 summarizes available recent seed crop data from 2007 to 2010 for Washington for *Brassica* and related vegetables. While the reports used to create Attachment Table 1 were not available for all *Brassica* and related crops reported to be grown in Washington, the acreage of crops for which reports are not available is likely relatively small. A 2010 summary reported a total of 2,829 acres of vegetable seed crops in western Washington in 2009 (McMoran, 2010). Of this, 2,369 acres (84%) were accounted for as cabbage, spinach and beet seed in Skagit County, leaving only 460 acres of all other seeds in Skagit County and other western Washington counties. A 1997 report of *Brassica* and related seed production in western and eastern Washington listed more crops than are shown in Attachment Table 2, but the totals were similar to those shown in the table (maximum 2,313 acres in eastern Washington and 2,160 acres in western Washington for an overall total maximum of 4,473 acres) (Thomas, 1997).

McMoran (2010) and du Toit (2007) both report that Washington produces 75% of the U.S. cabbage seed crop. Thomas et al., (1997,) reported that Washington produced 100% of the U.S. Brussels sprouts and kale seed crop, 75% of the U.S. cauliflower seed crop, and 70% of the U.S. radish seed crop. In addition Thomas et al. (1997) reported that the Washington Chinese cabbage, Chinese kale, and Chinese mustard seed crops represented 90-100% of the U.S. total.

In Oregon the 100-mile long Willamette Valley has approximately 900,000 acres of crops, of which more than 40% is grass and legume seed production; most of the remaining acreage is in grains, hay and forage (OSU 2011). While there are other seed production areas in Oregon, the Willamette Valley is the major vegetable seed production area. Approximately 13,000 acres or 1.4% of total acres are reported for vegetable and flower seed production (OSU, 2011).

According to the Willamette Valley Specialty Seed Association (WVSSA), common *Brassica* and related seed crops in the Willamette Valley are: cabbage, Brussels sprouts, turnip, rutabaga, radish, Chinese cabbage, Oriental brassicas, mustard, and kale (WVSSA, 2011). According to Mallory-Smith, et al. (2007) western Washington and western Oregon combined produce nearly

Attachment Table 1. Washington State Brassica and Related Vegetable Seed Production

		West of Cascades (Western)								Columbia Basin (Eastern)		
	Acres	**Skagit**	**Island**	**Jefferson**	**Snohomish**	**Clallam**	**Lewis**	**San Juan**	**Whatcom**	**Grant**	**Franklin**	**Adams**
Cabbage (*B. oleracea*)	600	√	√		√	√		√				
Cauliflower (*B. oleracea*)	100	√			√	√		√	√			
Collard (*B. oleracea*)	100-150	√							√	√		√
Kale (*B. oleracea*)	100-150						√			√		√
Kohlrabi (*B. oleracea*)	25-50	√								√		√
Chinese kale (*B. alboglabra;* or *B. oleracea ssp. alboglabra)*	< 100	√							√	√		√
Rutabaga (*B. napus*)	80	√			√							
Chinese mustard (*B. rapa*)	350	√							√	√		√
Turnip (*B. rapa*)	400	√							√	√		√
Arugula (*Eruca sativa*)	100-120	√				√			√	√		√
Daikon (*Raphanus sativus*)	400-500									√		√
Radish (*Raphanus sativus*)	1500-2000	√			√					√	√	√
TOTAL FROM RECENT REPORTS	3,750-4,500											

Source: WSU, 2010.

all (~90%) of European cabbage, Brussels sprouts, rutabaga and turnip seed, and a substantial portion (20-30%) of radish, Chinese cabbage and other Asian *Brassica* vegetable crops seed.

California and Arizona Production.

The California Pest Management Center's (CPMC, 2011) crop management database reports the following *Brassica* and related vegetable seed crops by region, but with no acreage information:

- Region 2 - Central Valley: broccoli, Chinese cabbage, cabbage, cauliflower, kale, kohlrabi, radish, rutabaga and turnip.
- Region 3 – central coast: broccoli, cabbage, cauliflower, kale, kohlrabi, mustard and radish.
- Region 4 – southeast, bordering Nevada and Arizona; includes Imperial Valley – broccoli, cabbage, cauliflower, collard greens, kale, kohlrabi, mustard, radish and rutabaga.
- Region 5 – south coast (Santa Barbara to Mexican border): broccoli, cabbage, cauliflower, collard greens, kale, kohlrabi, mustard, and radish.
- Region 6 – northern Central Valley – broccoli, Chinese cabbage, cabbage, cauliflower, collard greens, kale, kohlrabi, mustard and radish.

In regions 2 and 4 canola is grown, but there is no acreage information.

California and Arizona are the only states for which specific mention of broccoli seed production was found. In 2003 the University of Arizona Extension reported 4,500 acres of seed crops in Yuma County, but did not specify the seed types (University of Arizona, 2003).

A seed company in the Yuma Valley reports it specializes in hybrid broccoli seed production (SeedQuest, 2011). It also offers cauliflower, Chinese cabbage (pak choi), mustard, and Asian types of radish.

Idaho Production

Radish seeds are reportedly produced in Idaho's Treasure Valley, which is located in western Idaho and is the lower valley of the Snake River (OSA, 2007).

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Appendix J: Herbicide Resistance

J.1. Introduction

Herbicides are pesticides intended to prevent or kill weeds that can compete with a crop for nutrients, water and in some cases, sunlight. By killing weeds, herbicides allow planted crops to grow and thrive, thereby increasing crop yield and allowing these crops to be grown on fewer acres, protecting habitat and wildlife from unnecessary expansion of cropland production wherever possible.

Plant populations can develop resistance to a herbicide due to the selection of individuals that carry altered genetic code(s) producing alleles that can render those individuals tolerant to the lethal effects of a herbicide. The application of a herbicide to the plant does not, itself, cause a mutation in subsequent generations. Rather, over time, those few plant biotypes that are not susceptible to a herbicide increase in number in the population with repeated use of the herbicide in the absence of other control methods, such as use of other chemical or mechanical control methods. The development of resistant populations is common to all herbicides. The probability for resistance to develop in a population is a function of: frequency of resistant allele(s), mechanism of resistance, dominance or recessive nature of the resistant allele(s), relative fitness of the resistant biotype and frequency of herbicide use in the absence of other control methods (Jasieniuk et al., 1996). The probability of resistance is not the same for all herbicides with some herbicides (e.g., ALS and ACCase classes) exhibiting resistance more quickly than other herbicides (e.g. glyphosate, auxins (dicamba), dinitroanilines).

Herbicide resistance could become a limiting factor in crop production if the resistant weed population cannot be controlled with other herbicides or cultural practices. This generally has not been the case for any herbicide and weed combination. For most crops, there are multiple herbicide options for growers to use. However, good management practices to delay the development of herbicide resistance have been identified, are being actively promoted by the public and private sectors, and are being implemented by growers.

Monsanto considers product stewardship to be a fundamental component of customer service and business practices. Stewardship of the glyphosate molecule to preserve its usefulness for growers is an important aspect of Monsanto's stewardship commitment. Although herbicide resistance may eventually occur in weed species when a herbicide is widely used, resistance can be delayed, contained and managed through research, education and good management practices. Monsanto will invest in research to identify best management practices, monitoring for resistance, and in dealer/grower education and training programs to provide information on best practices to manage glyphosate weed resistance in canola. These are the key elements of Monsanto's approach to providing stewardship of glyphosate relative to all uses including use in the MON 88302 canola production system. This document provides an overview of resistance to glyphosate and Monsanto's approach to the development of best management practices to mitigate glyphosate resistance.

J.2. The Herbicide Glyphosate

Glyphosate (N-phosphonomethyl-glycine) (CAS Registry #: 1071-83-6), the active ingredient in the Roundup family of nonselective, foliar-applied, post-emergent agricultural herbicides, is among the world's most widely used herbicidal active ingredients. Glyphosate is highly effective against the majority of economically significant annual and perennial grasses and broadleaf weeds. Currently, glyphosate is labeled for control of more than 300 weed species world-wide. Glyphosate kills plant cells by inhibition of 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), an enzyme involved in the shikimic acid pathway for aromatic amino acid biosynthesis in plants and microorganisms (Franz et al., 1997; Steinrücken and Amrhein, 1980). Glyphosate is the only known herbicide with this mode of action (Franz et al., 1997). The relevant aromatic amino acid pathway is not present in mammalian metabolic systems (Cole, 1985; Franz et al., 1997). A comprehensive human safety evaluation and risk assessment concluded that glyphosate has low toxicity to mammals, is not a carcinogen, does not adversely affect reproduction and development and does not bioaccumulate in mammals (Williams et al., 2000). An ecotoxicological risk assessment concluded that the use of glyphosate does not pose an unreasonable risk of adverse effects to nontarget species, such as birds and fish, when used according to label directions (Giesy et al., 2000). Glyphosate has favorable environmental characteristics, including a low potential to move through the soil to reach ground water and is degraded over time by soil microbes. Because it binds tightly to soil, glyphosate's bioavailability is reduced immediately after application, which is why glyphosate has no residual soil activity. Similar conclusions were reached in an independent assessment of risk to human health and to the environment that was conducted as a part of the Environmental Impact Statement for Glyphosate Tolerant Alfalfa (USDA-APHIS, 2010).

J.3. Herbicide Use in Canola Systems and Herbicide-resistant Weeds

Weed control in canola production fields is critical for obtaining optimized yields. Because failure to control weeds within the crop can result in decreased yields and reduced crop quality, a program for weed control is essential to ensure a grower can optimize yields. In using MON 88302 weed control will be obtained through the use of glyphosate plus other herbicides. Control of weeds in a crop is essential because weeds compete with the crop for the same limited resources in the field, including sunlight, water and nutrients. Weed competition can be a major limiting factor in canola production leading to significant yield reductions (CCC, 2006). Certain perennial weeds, such as Canada thistle, are known to be particularly important to control in canola production. For example, studies have demonstrated that only 10 Canada thistle plants per square meter have resulted in 10% yield loss while 40 plants per square meter have resulted in over 50% yield loss (CCC, 2006). Glyphosate is highly effective against the majority of annual and perennial grasses and broad-leaf weeds including difficult-to-control weeds such as Canada thistle (NDSU, 2005; Padgette et al., 1996).

With any herbicide use, however, comes the potential for the selection of weeds resistant to that herbicide. Within a weed species, individuals may possess an inherent ability to withstand the effects of a particular herbicide. Repeated use of that herbicide will expose

the weed population to a "selection pressure," which may lead to an increase in the number of surviving resistant individuals in the population (Jasieniuk et al., 1996). In other words, plants susceptible to the applied herbicide will die, while those few having some type of natural resistance may survive and reproduce.

A resistant weed must demonstrate two criteria as defined by the Weed Science Society of America website at www.wssa.net: (1) the ability to survive application rates of herbicide product that once were effective in controlling it and; (2) resistance is heritable. Herbicide-resistant weeds are neither a new phenomena nor is resistance unique to glyphosate. Growers have been managing herbicide-resistant weeds for decades with the use of alternative herbicides and/or cultural methods such as tillage or crop rotation that are combined to provide a diverse weed management program.

J.4. Characteristics of Herbicides and Herbicide Use Influencing Resistance

While the incidence of weed resistance is often associated with repeated applications of a herbicide product, the actual onset of resistance within a population depends very much on the specific herbicide chemistry in question, as well as the inherent presence of gene(s) that confer the ability of a plant to be resistant to a particular chemical within a specific weed species and even a specific population of that species (Sammons et al., 2007). Some herbicide products are much more prone to develop herbicide resistance than others (Heap, 2009). Considering the substantial worldwide glyphosate-treated acreage, and the total number of weeds that glyphosate can control, glyphosate, a member of the glycine herbicide chemical family, has been used extensively for over three decades with relatively few cases of resistance development, particularly when compared to many other herbicides (e.g., ALS inhibitors, triazines, and ACCase inhibitors). The graph in Figure J-1 illustrates the instances of weed resistance to various herbicide groups. The different slopes observed are largely due to the factors described above, which relate to chemistry and function, in addition to levels of exposure in the field. The summary below describes herbicide-specific factors determined to be important in the process of selecting for individuals that are inherently resistant to a herbicide.

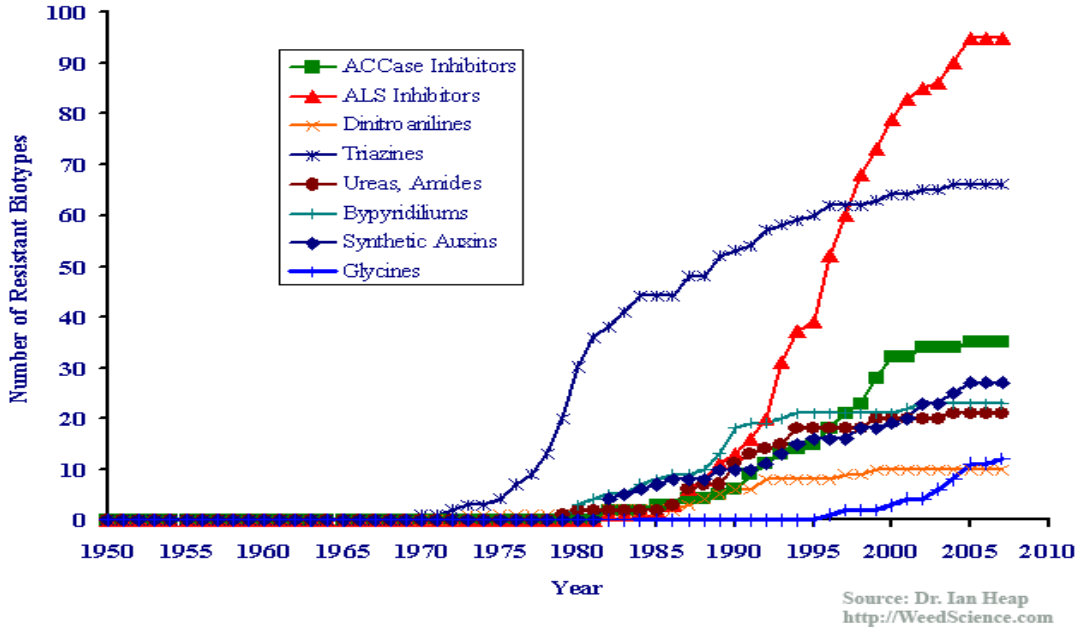

Figure J-1. Weed Resistance to Various Herbicide Chemical Families

J.5.1. Mechanisms of Resistance

The application of a herbicide to a weed does not, itself, cause a mutation in later generations of the plant. Rather, over time, the repeated application of herbicide selects for those few biotypes that are less susceptible to the herbicide and they increase in frequency within a population. To date, the three known mechanisms by which a weed species develops resistant to a herbicide have been identified as 1) target site alteration (target site); 2) enhanced metabolism of the herbicides (metabolism); and 3) reduced absorption and/or translocation of the herbicide such that the herbicide does not get to the site of action within the plant cell (exclusion) (Sammons et al., 2007).

Herbicide resistance via target site is the most common resistance mechanism among the various herbicide classes. It has been found that a target site mechanism is the most common mechanism for ALS inhibitors, ACCase inhibitors, and triazines, but is less common for glyphosate. One type of target site alteration involves amino acid substitution(s) in the enzyme that prevents the binding of the herbicide without impacting enzyme activity, and the plant is able to grow normally without any fitness penalty. For ALS inhibitors, the level of resistance conferred by a target site mechanism has been found to be as high as 3,400 X (Ferguson et al., 2001). (Note: X is the labeled or recommended rate for a herbicide on a particular weed species.) For glyphosate, species found to exhibit a target site mechanism can demonstrate low levels of resistance (2-3X) due to the fact that glyphosate is a true transition state inhibitor (Sammons et al., 2007; Schönbrunn et al., 2001) differentiating glyphosate from ALS inhibitors and ACCase inhibitors. The transition state is an unstable state during an enzyme reaction half-way between the substrate and product. Transition state inhibitors are very effective enzyme inhibitors. In addition, multiple alterations of the same enzyme have been found for ALS inhibitors (Tranel and Wright, 2002). This may explain the apparent high frequency of resistance and the short time in which resistance developed to herbicides in these two classes of chemistries. Only one altered site in the targeted plant EPSPS enzyme impacting glyphosate insensitivity has been found for glyphosate (Baerson et al., 2002). Another type of a target site resistance mechanism recently discovered for glyphosate, is an over amplification of the EPSPS gene which results in an overproduction of the EPSPS enzyme (Gaines et al., 2010). This mechanism was discovered in Palmer pigweed.

The second general type of herbicide resistance mechanism, metabolism, has not been found to be a resistance mechanism associated with glyphosate in any of the weed species studied thus far. However, legumes have been shown to degrade glyphosate and therefore this type of resistance mechanism may be active in some species (Gaines et al., 2010).

Herbicide resistance as a result of exclusion mechanisms is the glyphosate resistant mechanism among the majority of the weed species studied to date. This resistance mechanism has also been found to be associated with 2,4D and paraquat. Within this category, there are two types of translocation alterations that have been observed for glyphosate; (a) restricted movement of glyphosate from leaf cells into the meristematic cells of the plant (Shaner, 2009) and (b) decreased delivery of glyphosate to the

chloroplast (Sammons et al., 2007). The level of glyphosate resistance conferred with this mechanism is higher (6-8X) than for species exhibiting amino acid substitution type target site mutations (2-3X).

In some species, the experimental evidence suggests that more than one mechanism of glyphosate resistance may occur within the same plant to protect the plant from the phytotoxic effects of glyphosate (Yu et al., 2007). This implies that multiple genes (polygenic resistance) are necessary and thus the selection of plants with multiple genes needed to confer resistance would be expected to occur at a low frequency.

In summary, the overall low occurrence of glyphosate resistance may be in part explained by: (1) the nature of the target site inhibition by glyphosate relative to other herbicides; (2) the lack of metabolism as a mechanism of selectivity for weed resistance, and (3) evidence of multiple mechanisms being necessary for resistance; thus, resistance is polygenic and difficult to assemble and maintain. Recommendations to manage glyphosate resistance are not dependent upon the type of resistant mechanism operating within a species or population of a species.

J.5.2. Use of Recommended Rate

The interaction between herbicide application rate and resistance for post-emergence herbicides, such as glyphosate, is dependent upon the nature of the plant gene(s) conferring resistance to the chemical. In general, herbicide rate has more effect on selecting for resistant individuals in a population if the resistant gene is semi-dominant or recessive as compared to the resistant gene being dominant. Likewise, herbicide rates would have more of an effect on the onset of resistance if commercially significant resistance required the additive effect of multiple genes (i.e. quantitative or polygenic resistance). Low rates would tend to allow certain biotypes to survive and mate with other biotypes of the same or an alternate resistant gene. The offspring of this mating may then be able to survive a full rate.

Less-than-recommended or suboptimal rates have been implicated as or speculated to be the causal factor in herbicide resistance for several different weed species, including chlortoluron-resistant blackgrass, diclofop-resistant ryegrass and dicamba-resistant kochia (Beckie, 2006). It has been demonstrated that, in three generations of a ryegrass biotype sprayed at sublethal rates of diclofop-methyl or glyphosate, a high level of resistance to diclofop-methyl and a moderate level of resistance to glyphosate evolved (Busi et al., 2009). The conclusion of this research was that growers should avoid lowering the application rate of herbicides.

J.6. Weeds Resistant to Glyphosate

As with any other herbicide, the use of glyphosate may lead to the development of glyphosate-resistant weed species. A list of glyphosate resistant weeds is provided below in Table J-1. However, the potential for the development of a glyphosate-resistant weed needs to be considered in the following context: 1) if a glyphosate-based weed control system was not available, other herbicide(s) with equal or greater potential for resistance

would be used to control weeds and 2) other herbicides and cultural practices can be used to manage the glyphosate resistant species (Gustafson, 2008; Neve, 2008).

Through March 2011, biotypes of twenty-one weed species resistant to glyphosate have been identified and confirmed worldwide (WSSA, 2011a). Twelve species resistant to glyphosate have been confirmed in the U.S., three of which were identified outside of Roundup Ready cropping systems. The speed of spread and geographical distribution of the resistant species has varied. Some species with resistant biotypes, such as common ragweed (*Ambrosia artemisfolia*), have been found in a limited number of sites across the mid-west, whereas marestail (*Conyza canadensis*) has been found in many states in the northeast, mid-west and the south. The reproductive biology of the particular weed species involved appears to be a factor contributing to the spread of resistant biotypes. In the above examples, marestail produces a large number of wind-dispersed seeds, which contributes to rapid spread (Weaver, 2001), while ragweed seeds do not have features that allow for such easy distribution by the wind.

Table J-1. U.S. Glyphosate Resistant Weeds through March 2011

Weeds identified outside of Roundup Ready Systems	Rigid ryegrass (*Lolium rigidum*) Annual bluegrass (*Poa annua*) Hairy fleabane (*Conyza bonariensis*)
Weeds identified in Roundup Ready Systems	Horseweed (*Conyza canadensis*) Common ragweed (*Ambrosia artemisiifolia*) Giant ragweed (*Ambrosia trifida*) Palmer amaranth (*Amaranthus palmeri*) Common waterhemp (*Amaranthus rudis*) Italian ryegrass (*Lolium multiflorum*) Johnson grass (*Sorghum halepense*) Kochia (*Kochia scoparia*) Goosegrass **(***Eleusine indica***)**

Some weed species, such as *Equisetum arvensis* (field horseweed), are tolerant, as opposed to resistant, to glyphosate. That is, they have the inherent ability to survive herbicide treatments as opposed to resistance induced by such techniques as genetic engineering or selection of variants produced by tissue culture or mutagenesis (WSSA, 2011b). Some species are more difficult to control with glyphosate than others (e.g. lambsquarters (*Chenopodium album*) and morninglory (*Ipomea* sp.)) and require more care to make sure the correct amount of glyphosate is applied at the right growth stage. For these difficult-to-control weeds, environmental conditions can affect herbicide performance more than for weeds that are easier to control, and therefore it is more critical that the correct rate be applied at the right growth stage when making applications to weeds in the difficult-to-control category. Weed control situations involving tolerant or difficult-to-control species are often confused with resistance.

J.7. Use of Glyphosate for In-crop Weed Management

Monsanto has developed plants through biotechnology to be tolerant to glyphosate. The development, approval and cultivation of these Roundup Ready crops have facilitated additional uses of glyphosate in crops where such uses were not previously possible given the non-selective nature of glyphosate. This development has provided growers with an additional weed management option and benefits relative to existing weed management options. The glyphosate tolerance in Roundup Ready crops has no effect per se on the control of weeds. From a weed resistance standpoint, the use of glyphosate with glyphosate-tolerant canola is no different than the use of a selective herbicide in a conventional canola crop.

The most often cited benefits of glyphosate as an in-crop weed management option are simplicity, flexibility of application timing, weed spectrum, crop safety and environmental safety (Dill, 2005). The ability to use glyphosate in-crop has allowed farmers to change their farming practices in some cases. For example, planting of Roundup Ready cotton, corn and soybean have resulted in an increase in no-tillage practices (Dill et al., 2008).

Since Monsanto commercialized the first Roundup Ready canola varieties in 1998, growers have enthusiastically adopted the technology. The Roundup Ready canola system, (i.e., planting Roundup Ready canola and applying glyphosate in-crop), has become the standard weed control program in U.S. canola production. In addition, weed control in a Roundup Ready canola system likely will involve not only glyphosate-based herbicides but also other herbicides and weed management practices to effectively manage weeds, thus increasing crop yield and reducing development of resistant weed populations. State Universities/Cooperative Extension Services (CES) publish information on best weed management practices in Roundup Ready crops to address both of these objectives (see Table J-2). In addition Monsanto and other companies selling glyphosate products provide information on these same best management practices as detailed later in this Appendix.

J.8. Weed Resistance Management Strategies for Glyphosate

As part of Monsanto's stewardship of Roundup agricultural herbicides and Roundup Ready crop systems, the company has conducted investigations and worked extensively with academics and other herbicide manufacturers to understand the best practices to manage resistance. These investigations have demonstrated that one of the major factors that can contribute to the development of resistant weed populations is weed control management practices such as the application of herbicides at rates below those indicated on the EPA-approved label for the weed species (Beckie, 2006), and sole reliance on a particular herbicide for weed control without the use of other herbicides or cultural control methods (i.e. pre-plant and in-crop tillage) over an extended period of time (Beckie, 2006; Peterson et al., 2007).

As detailed in the Petition and Appendix I, the purpose of MON 88302 is to provide growers with improved weed control through greater flexibility of glyphosate herbicide

application. By virtue of enhanced CP4 EPSPS expression in male reproductive tissues, MON 88302 provides tolerance to glyphosate during the sensitive reproductive stages of growth, and enables the application of glyphosate at higher rates and at later stages of development than is possible with the current product.

Monsanto will communicate to all canola growers recommended weed resistance management practices. Monsanto will provide instructions to canola growers regarding reporting any incidence of repeated non-performance of Roundup agricultural herbicides on a particular weed, and Monsanto will investigate cases of unsatisfactory weed control to determine the cause of poor performance. In cases where resistance is confirmed, Monsanto will provide recommendations for alternative control methods for farmers (see Table J-2). These recommendations are made available through Monsanto supplemental labels, the Monsanto Technology Use Guide (TUG), Monsanto and University publications and internet sites for growers, consultants, retailers and distributors. In all cases of glyphosate-resistant weeds in the U.S. and globally, there are alternative herbicides and cultural methods available to farmers to effectively control these species. Some examples of these recommendations from University/CES personnel are found in Table J-2. It is important to note that there are many alternative options in each situation.

The weed resistance management recommendations that will be made for the use of glyphosate in conjunction with varieties containing the second generation glyphosate-tolerant MON 88302 event will not differ from recommendations being made for commercial varieties containing event RT73 (Roundup Ready canola varieties). These recommendations are consistent with the Herbicide Resistance Action Committee's guidelines for prevention and management of herbicide resistance (HRAC, 2011). These guidelines recommend an integrated approach to weed resistance management including crop management (i.e. row spacing, etc), cultural techniques and herbicides. The EPA is the U.S. federal regulatory agency that administers the federal law governing pesticide sale and use (FIFRA). The U.S. EPA encourages pesticide manufacturers to provide growers with information regarding a herbicide's mode of action to aid growers in planning herbicide use practices and to foster the adoption of effective weed-resistance management practices as specified by the U.S. EPA in PR Notice 2001-5. In that document the U.S. EPA states that "this approach to resistance management is sound and would be highly beneficial to pesticide manufacturers and pesticide users" (U.S. EPA, 2001). The U.S. EPA approves all pesticide label use instructions based on the agency's evaluation of supporting data supplied by the pesticide registrant or manufacturer. After the U.S. EPA approves a pesticide label, it is a violation of federal law to use the pesticide for a use or in a manner not in accordance with the label directions.

Monsanto incorporates the U.S. EPA's guidelines for pesticide resistance management labeling on its glyphosate-based agricultural herbicide labels, and will do so on the label for products to be applied over the top of varieties developed containing MON 88302 (An example of the current Roundup WeatherMAX product label is available at http://www.cdms.net/LabelsMsds/LMDefault.aspx?manuf=23&t). The U.S. EPA-approved labels for Roundup branded herbicide weed-resistant management recommendations are designed to minimize the potential for the development of glyphosate-resistant weeds. By approving a label for a glyphosate-based agricultural

herbicide, the U.S. EPA has concluded that the product will not cause unreasonable adverse effects to the environment or human health when used in accordance with the label's directions.

The weed resistance management guidelines on the labels of Roundup agricultural herbicides include recommendations that are well-documented in the scientific literature as being appropriate and effective for weed control, and that mitigate weed resistance. Significant research has been conducted to identify the appropriate application rate of glyphosate required to control a particular weed at various growth stages under various agronomic and environmental conditions. These rates are based on over 35 years of ongoing research at Monsanto to evaluate the efficacy of Roundup agricultural herbicides. Studies have included efficacy of weed control for a broad spectrum of weeds and under a wide range of conditions. A key element of effective weed control and weed resistance management, therefore, is using the correct rate of glyphosate at the right time for the weed species and the size of the weed (i.e., using a lethal dose which avoids the need for subsequent applications). This important strategy is well-supported by field research studies at several universities (Westra et al., 2008). Additionally, it is accepted in the weed science community that the use of multiple herbicide modes of action via tank mixtures, use of herbicides with different modes of action in a rotational crop, or using multiple herbicides in sequence within a crop will reduce the risk of developing weed resistance (Beckie, 2006; Gressel and Segel, 1990). Tank-mixing involves mixing two or more herbicides in the spray tank immediately prior to application. To provide growers with the tools needed to minimize resistant weed development, Monsanto will continue to investigate and recommend appropriate residual and post-emergence herbicide products that have a different mode of action from glyphosate. As an example, the herbicide clopyralid (tradename Stinger) is a post-emergence herbicide that will help control flushes of annual broadleaf seed which could slow the selection and potential spread of glyphosate-resistant weeds in Roundup Ready systems. The general concept that Monsanto promotes for management of resistance has been referred to by several authors as applying "diversity" across cropping/fallow seasons to manage weed resistance (Beckie, 2006; Powles, 2008). Crop rotation and management of the fallow period and cover crops can be important considerations in managing resistance.

Table J-2. Management Recommendations for Control of Glyphosate Resistant Weeds in Canola and Rotational Crops

Glyphosate Resistant Weed	Crop	Recommendations for alternative herbicides to manage glyphosate resistant weeds[1]	Reference[2]
Palmer amaranth	Canola	PPI: trifluralin, ethalflurafin Post: glufosinate(LL canola)	ND
	Maize	PP: 2,4-D, paraquat Pre: alachlor, alachlor+atrazine Post: 2,4-D, dicamba, atrazine	AR and GA
	Soybean	PP: flumioxazin, metribuzin/chlorimuron Pre: flumioxazin, metolachlor/metribuzin Post: fomesafen, metolachlor/fomesafen	AR and GA
	Wheat/Barley	PP: paraquat, 2,4-D, dicamba Pre: saflufenacil Post: fluroxypyr/florasulam, pyrasolfotole/bromoxynil, 2,4-D	ND, GA, Product Labels
Waterhemp	Canola	PPI: trifluralin, ethalflurafin Post: glufosinate(LL canola)	ND
	Maize	PP: 2,4-D, atrazine Pre: acetochlor, acetochlor+atrazine, topramezone, tembotrione Post: mesotrione, atrazine, 2,4-D+atrazine	OH/IN
	Soybean	PP: metribuzin/chlorimuron Pre: metolachlor/metribuzin, sulfentrazone, flumioxazin Post: lactofen, fomesafen, acifluorfen	OH/IN
	Wheat/Barley	PP: paraquat, 2,4-D, dicamba Pre: saflufenacil Post: fluroxypyr/florasulam, pyrasolfotole/bromoxynil, 2,4-D	ND
Common ragweed	Canola	Post: clopyralid, glufosinate(LL canola)	ND
	Maize	PP: 2,4-D, dicamba, atrazine Pre: acetochlor, atrazine, rimsulfuron/isoxaflutole, saflufenacil Post: 2,4-D, dicamba, atrazine, mesotrione/atrazine	OH/IN
	Soybean	PP: 2,4D, chlorimuron, metribuzin/chlorimuron Pre: sulfentrazone/chloransulam, chloransulam/flumioxazin, Post: cloransulam, fomesafen	OH/IN
	Wheat/Barley	PP: 2,4-D, dicamba, paraquat Pre: saflufenacil Post: 2,4-D, dicamba, bromoxynil	ND

Table J-2. Management Recommendations for Control of Glyphosate Resistant Weeds in Canola and Rotational Crops (continued)

Glyphosate Resistant Weed	Crop	Recommendations for alternative herbicides to manage glyphosate resistant weeds	Reference[2]
Giant ragweed	Canola	Post: clopyralid, glufosinate(LL canola)	OH/IN
	Maize	PP: 2,4-D, dicamba Pre: atrazine, saflufenacil, Post: 2,4-D, dicamba, clopyralid, mesotrione/atrazine	OH/IN
	Soybean	PP: 2,4-D Pre: metribuzin/chlorimuron, cloransulam, sulfentrazone/chlorimuron, flumioxazin/cloransulam Post: fomesafen, lactofen	OH/IN
	Wheat/Barley	PP: 2,4-D, dicamba, bromoxynil Pre: saflufenacil Post: 2,4-D, dicamba, bromoxynil	OH/IN, Product Labels
Marestail	Canola	PP: 2,4-D, dicamba Post: glufosinate(LL canola)	ND, NE
	Maize	PP: 2,4-D+dicamba, atrazine+2,4-D Pre: mesotrione, saflufenacil, rimsulfuron/isoxaflutole Post: 2,4-D+dicamba, clopyralid	OH/IN, ND
	Soybean	PP: 2,4D, 2,4-D+metribuzin/chlorimuron Pre: sulfentrazone/chloransulam, saflufenacil, flumioxazin, metribuzin, saflufenacil Post: cloransulam, fomesafen	OH/IN, ND
	Wheat/Barley	PP: 2,4-D, dicamba+MCPA Pre: saflufenacil Post: 2,4-D, pyrasolfotole/bromoxynil, dicamba/fluroxypyr	ND
Kochia	Canola	Pre: ethalflurafin Post: glufosinate(LL canola)	ND
	Maize	PP: dicamba, bromoxynil, atrazine Pre: atrazine, saflufenacil, sulfentrazone Post: dicamba, bromoxynil, fluroxpyr	ND and NE
	Soybean	PP: flumioxazin Pre: sulfentrazone, clomazone, saflufenacil, flumioxazin/chlorimuron Post: chlorimuron, fomesafen, imazethapyr	ND and NE
	Wheat/Barley	PP: dicamba, bromoxynil, paraquat Pre: saflufenacil Post: dicamba, bromoxynil, tribenuron	ND

[1] Application Timings: PP = Preplant burndown before planting; Pre = pre-emergence; Post = post-emergence; Post-directed = applied post-emergence directed at the base of the crop; PPI = Pre-plant incorporated.
[2] References: AR: (University of Arkansas, 2011); GA: (University of Georgia, 2010); TN: (University of Tennessee, 2010); OH/IN: (OSU, 2011); NE: (University of Nebraska-Lincoln, 2011); ND: (Zollinger, 2010).

J.9. Monsanto Weed Performance Evaluation and Weed Resistance Management Plan

Monsanto and/or Monsanto seed company licensees are directly in a position to be aware of the performance of glyphosate in all fields through its extensive presence in the markets where Roundup Ready canola is grown, through its relationship with farm advisors, and its relationship with key University/CES personnel. This will allow the timely recognition of performance issues that could arise related to weed resistance or other means. Monsanto field employees and hired consultants are trained and provided processes for responding to product performance inquiries. As warranted, individual performance issues that could be related to potential resistance are promptly handled. In addition, performance inquires are periodically reviewed for trends that could indicate the need for follow up action on a broad scale. If broad scale actions in the areas where canola is produced are needed, Monsanto and/or licensees will alert the canola growers of the need for any prescribed action.

In general, when resistance is confirmed, the scientific and grower communities are notified and a weed resistance mitigation plan is implemented by Monsanto in cooperation with the University/CES. The mitigation plan is designed to manage the resistant biotype through effective and economical weed management recommendations implemented by the grower. The scope and level of intensity of the mitigation plan may vary depending on a combination of the following factors: 1) biology and field characteristics of the weed (seed shed, seed dormancy, etc.); 2) importance of the weed in the agricultural system; 3) resistance status of the weed to other herbicides with alternate modes of action; and 4) availability of alternative control options. These factors are analyzed by Monsanto and University/CES personnel in combination with economic and practical management considerations to develop a tailored mitigation strategy. The plan considers what is technically appropriate for the particular weed and incorporates practical management strategies that can be implemented by the grower.

After a mitigation plan is developed, Monsanto communicates the plan to the grower community through the use of supplemental labeling (labeling which includes newly approved uses, use directions or other instructions which have been added since the last EPA-approved Master label), informational fact sheets, retailer training programs, agriculture media and/or other means, as appropriate.

In addition to the grower inquiry initiated process, Monsanto, alone and in cooperation with University/CES, conducts field studies to understand the potential for weed resistance and weed shifts as the result of various weed management programs implemented in a Roundup Ready canola system. These studies allow researchers to better track specific factors that can influence the development of resistance to specific weeds.

J.10. Summary

Development of weed resistance is a complex process. No single agronomic practice will mitigate resistance for all herbicides or all weeds. As a result, weed resistance needs to be managed on a case-by-case basis and tailored for the particular herbicide and weed in order to meet grower needs. Using good weed management principles, built upon achieving high levels of control through proper application rate, choice of cultural practices, and appropriate companion weed control tools will allow Roundup agricultural herbicides to continue to be used effectively. In cases where weed populations have developed resistance to glyphosate, effective management options are available and experience has shown that growers continue to find value in using glyphosate in their weed control programs.

The key principles for effective stewardship of glyphosate use, including Roundup Ready crops, include: 1) basing weed management and weed resistance management practices on local needs and using the tools necessary to optimize crop yield; 2) using proper rate and timing of application; 3) not relying solely on one herbicide weed control option across a cropping system; 4) responding rapidly to instances of unsatisfactory weed control; and 5) providing up to date weed management and weed resistance management training.

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Appendix K: Potential Impact of Glyphosate on Human Health and the Environment

K.1. Overview

Glyphosate is a herbicide approved for use (registered) by the U.S. Environmental Protection Agency (EPA) for the control of weeds that would interfere with the growth of many food and non-food crops, including biotechnology-derived crops, as well as for control of weeds growing in non-crop areas. Glyphosate received a label for use in cropping systems, and has had food and feed tolerances for residues in the U.S. since 1975. In 2001, the U.S. U.S. identified glyphosate as the most widely used conventional agricultural herbicide in the U.S. (Kiely et al., 2004).

A comprehensive database on glyphosate has been evaluated by the EPA to support all currently approved uses. The EPA has repeatedly stated that it has a high level of confidence in the quality of the existing studies and the reliability of the toxicity endpoints that are the basis for human health and environmental risk assessments ((U.S. EPA, 2006a; 2006b). In establishing food and feed tolerances to support the use of glyphosate on animal feed and forage crops, the U.S.EPA concluded, "that there is a reasonable certainty that no harm will result to the general population, and to infants and children from aggregate exposure to glyphosate residues" (U.S. EPA, 2006d).

The following discussion provides an overview of the regulatory and risk assessment processes applicable to glyphosate and all other agricultural use pesticides. Glyphosate has been approved by the EPA for a large number of food and feed uses, including uses associated with glyphosate-tolerant crops. Over 180 food and feed tolerances (40 CFR § 180.364) have been established for glyphosate in support of these uses. A complete listing of all U.S. glyphosate tolerances is provided in Attachment 2.

K.1.1. Pesticide Registration and Tolerance Setting

The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) requires that before sale or distribution of a pesticide in the U.S., a person or company must obtain a registration, or license, from the U.S. EPA. Before registering a new pesticide or a new use for a previously registered pesticide, the U.S. EPA must first ensure that the pesticide, when used according to its label directions, will not cause unreasonable adverse effects on the environment. In order to address this standard, the U.S. EPA must evaluate potential risks to humans and the environment, and may require applicants to submit more than 100 different scientific studies and tests conducted according to the U.S. EPA guidelines. According to the U.S. EPA, glyphosate is one of more than over 1000 active ingredients currently registered as pesticides, which are formulated into many thousands of pesticide products that are available in the marketplace.

The process of registering a pesticide is a scientific, legal and administrative procedure through which the U.S. EPA examines the ingredients of the pesticide; the particular site or crop on which it is to be used; the amount, frequency, method and timing of application and other conditions of its use; and storage and disposal practices. In

evaluating a pesticide registration application, the U.S. EPA assesses a wide variety of potential human health and environmental effects associated with use of the product.

The data required by the EPA are used to evaluate whether a pesticide has the potential to cause adverse effects on humans, wildlife, fish and plants (including endangered species and non-target organisms that the pesticide is not intended to act against). This includes potential human health and safety risks ranging from short-term toxicity to long-term effects such as cancer and reproductive system disorders. The registration applicant must also supply data addressing the pesticide's potential impact on surface water or ground water (which can result from leaching or runoff, for example).

The U.S. EPA also must approve the language that appears on each pesticide label. A pesticide product can only be used legally according to the directions for use on the labeling accompanying it at the time of sale. Following these directions carefully and precisely is necessary to ensure safe use as defined by FIFRA.

The initial registration of a pesticide is not the only opportunity an agency like the U.S. EPA has to evaluate its safety. The 1988 amendments to FIFRA authorized the EPA to conduct a re-registration program of pesticides first registered before November 1, 1984. The goal of the re-registration program was to ensure that these pesticides met current scientific and regulatory standards and could be declared "eligible" for re-registration. The results of the U.S. EPA's reviews are summarized in Re-registration Eligibility Decision (RED) documents. In 1993, the U.S. EPA produced a 291-page RED on glyphosate (U.S. EPA, 1993b), describing/listing the data on which it made a decision to re-register all existing uses of the pesticide, based on the pesticide having met the no unreasonable adverse effects standard described in FIFRA. As mandated by the Food Quality Protection Act of 1996, the U.S. EPA initiated the Registration Review program to periodically re-evaluate all registered pesticides to ensure that as changes in science, public policy, and pesticide use practices occur, products in the marketplace can still be used safely. The Registration Review process for glyphosate started in 2009 and is expected to be completed in 2015. During the Registration Review process, the U.S. EPA will be requesting additional data for glyphosate due to recent changes in FIFRA data requirements and will be updating the risk assessments for all currently registered glyphosate uses.

The EPA also sets tolerances (maximum pesticide residue levels or MRLs) for the amount of the pesticide that can legally remain in or on foods and feeds. The U.S. EPA undertakes this analysis under the authority of the Federal Food, Drug, and Cosmetic Act (FFDCA). Under the FFDCA, the U.S. EPA must find that such tolerances will be safe, meaning that there is a reasonable certainty that no harm will result from aggregate exposure to the pesticide chemical residue and other potential exposure routes. This finding must be made and the appropriate tolerance established before a pesticide can be registered for use on the particular food or feed crop in question. Several factors must be addressed before a tolerance can be established, including:

- the aggregate exposure from the pesticide (now including occupational exposure[41], exposure through diet, from using pesticides in and around the home, and from drinking water);

- the cumulative effects from exposure to different pesticides that produce similar effects in the human body;

- whether there is increased exposure to infants and children, or other potentially high exposure subpopulations; and

- whether the pesticide produces an effect in humans similar to an effect produced by a naturally occurring estrogen or produces other endocrine-disruption effects.

K.1.2. Pesticide Risk Assessment

The process the U.S. EPA uses for evaluating the health impacts of a pesticide, under either FIFRA or the FFDCA, is called a risk assessment. The EPA uses the National Research Council's four-step process for human health risk assessment, which involves hazard identification, dose-response assessment, exposure assessment and risk characterization. Each of these steps is discussed below:

Hazard Identification

The first step in the risk assessment process is to identify potential health effects, or hazards that may occur from different types of pesticide exposure. The U.S. EPA considers the full spectrum of a pesticide's potential health effects. Hazards are identified through a battery of studies that examine the potential toxicity of the pesticide in various tests including, where appropriate, tests with laboratory animals.

Generally, for human health risk assessments, many toxicity studies are conducted, based on EPA guidelines, by pesticide companies in independent laboratories following the Good Laboratory Practice (GLP) standards, and evaluated for acceptability by U.S. EPA scientists. The U.S. EPA evaluates pesticides for a wide range of effects, from eye and skin irritation to cancer and birth defects. The U.S. EPA may also consult the public literature or other sources of information on any aspect of the chemical.

Dose-response assessment

The next step of the risk assessment considers the levels at which the pesticide produces adverse effects. Dose-response assessment involves considering the dose levels at which adverse effects were observed in test animals, and using these dose levels to calculate an equal dose in humans.

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[41] Historically, issues associated with potential occupational exposure for each new use are were considered separately under FIFRA's unreasonable risk standard; however, under a recently announced revised policy, the EPA has stated that it intends to begin to include occupational exposure into the aggregate assessment (74 FR 65121, EPA–HQ–OPP–2009-0889; December 9, 2009).

Exposure Assessment

Step three of the process involves an exposure assessment. People can be exposed to pesticides in three ways: 1) inhaling pesticides (inhalation exposure); 2) absorbing pesticides through the skin (dermal exposure); and 3) ingesting pesticides (oral exposure). Depending on the situation, pesticides could enter the body by any one or all of these routes. Typical sources of pesticide exposure include agricultural (food); home and personal use pesticides; pesticides applied to lands that make their way into the drinking water; or occupational exposure for agricultural workers or pesticide applicators.

Risk Characterization

Risk characterization is the final step in assessing human health risks from pesticides. It is the process of combining the hazard, dose-response and exposure assessments to describe the overall risk from the use of a pesticide. It explains the assumptions used in assessing exposure as well as the uncertainties that are built into the dose-response assessment. The strength of the overall database is considered, and broad conclusions are made. The U.S. EPA's role is to evaluate both toxicity and exposure and to determine the risk associated with use of the pesticide.

The risk to human health from pesticide exposure depends on both the toxicity of the pesticide and the likelihood of people coming into contact with it (exposure). At least *some* exposure and *some* toxicity are required to result in a risk. For example, if the pesticide is found to have a high level of toxicity, but people are not exposed to the pesticide, there is no risk. Likewise, if there is ample exposure but the pesticide is essentially nontoxic, there is no risk. However, usually when pesticides are used, there is some toxicity and exposure, which results in a potential risk.

The U.S. EPA recognizes that effects of exposure to all pesticides including glyphosate vary between animals of different species (interspecies extrapolation) and from person to person (intraspecies variability). To account for this variability, a 100-fold *uncertainty factor* is built into the risk assessment (10X for interspecies extrapolation and 10X for intraspecies variability). This uncertainty factor creates an additional margin of safety for protecting people who may be exposed to the pesticides.

Once the risk assessment process for a pesticide is complete, the U.S. EPA uses this information to determine if (when used according to label directions) there is a reasonable certainty that the pesticide will not harm a person's health and will not cause unreasonable adverse effects on the environment.

Using the conclusions of a risk assessment, the U.S. EPA can then make a more informed decision regarding whether to approve a pesticide chemical or use, as proposed, or whether additional protective measures are necessary to limit occupational or non-occupational exposure to a pesticide. For example, the U.S. EPA may prohibit a pesticide from being used on certain crops because consuming that commodity treated with the pesticide may result in an unacceptable risk to consumers. Another example of protective measures is requiring workers to wear personal protective equipment (PPE)

such as a respirator or chemical resistant gloves, or not allowing workers to enter treated crop fields until a specific period of time has elapsed.

The U.S. EPA is responsible under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) to evaluate pesticides to ensure that they will not have unreasonable adverse effects on humans, the environment and non-target species when used according to label directions. Tier 1 "worst-case" or appropriately-refined higher tier risk assessments assist the U.S. EPA in determining whether or not risks would be below the U.S. EPA's levels of concern. Pesticides must be registered or exempted by the EPA's Office of Pesticide Programs before they may be sold or distributed in the U.S.

K.2. Potential Impact of Glyphosate on Human Health

Glyphosate presently has 186 established food and feed tolerances in the U.S. (see Attachment 2). Each time the U.S. EPA reviews an application to add a new food or feed use to the glyphosate label the Agency is required by FFDCA to conduct an aggregate risk assessment, considering all sources of human exposure to the pesticide, and find that aggregate exposure to the pesticide will be safe as defined by the statute and regulations. As noted above, historically, issues associated with potential occupational exposure were considered separately under FIFRA's unreasonable risk standard. However, under a recently announced revised policy, the U.S. EPA has stated that it intends to begin to include occupational exposure into the aggregate assessment (74 FR 65121, EPA–HQ–OPP–2009-0889; December 9, 2009).

Over the course of these numerous reviews the toxicology of glyphosate has been extensively studied. Multiple comprehensive toxicological studies in animals have demonstrated that glyphosate does not cause cancer, birth defects, mutagenic effects, nervous system effects or reproductive problems (EC, 2002; U.S. EPA, 1993b; WHO-FAO, 2004). In fact, after a thorough review of all available toxicology data, the EPA concluded that glyphosate should be classified in Group E carcinogen (evidence of non-carcinogenicity in Humans), the most favorable category possible (U.S. EPA, 1993a).

K.2.1. Glyphosate Safety Evaluations

Despite this extensive safety data, glyphosate safety is reviewed with every new use for which registration is sought, including, where necessary, uses associated with glyphosate-tolerant crops developed through biotechnology. As discussed above, prior to the approval of any new use of an existing registered pesticide, U.S. EPA must consider the potential human health effects from the aggregate (total combined) human exposure to that pesticide, combining the potential exposure from the proposed new use with all other existing exposures to the pesticide. Dietary exposure is considered, which addresses pesticide residues that may remain on food from crops on which the pesticide is applied (pre- or post-emergence), as well as any residue that could be found in drinking water as a result of pesticide use. Non-dietary exposure is also included in this assessment, which includes exposure to the pesticide through residential use, such as on lawns or in flower beds, as well as exposure in a recreational context, such as from a golf course or sports

field. Based on these data, the U.S. EPA must be able to make a determination of reasonable certainty of no harm to human health as required by the FFDCA.

The U.S. EPA does not conduct an acute dietary risk assessment for glyphosate because no acute human health concerns have ever been determined from toxicological studies conducted with glyphosate. Accordingly, the U.S. EPA does not expect glyphosate to pose an acute risk (U.S. EPA, 2006a; 2006b). The U.S. EPA assesses chronic dietary risk for glyphosate using "worst-case" (Tier 1) exposure assumptions that include tolerance level residues and 100% crop treated. For food, this estimate assumes that glyphosate is used on 100 percent of all the crops on which the pesticide is currently approved for use. It further assumes that the resulting pesticide residues found on all harvested food crops are at the level of the legally established tolerance (i.e., the maximum allowable pesticide residue level). For water, the U.S. EPA assumes that glyphosate is used to control weeds in water bodies by direct application to the water at the maximum application rate, without taking into account degradation in the water body or partitioning to sediment within the water column (U.S. EPA, 2006a; 2006b).

Applying this highly conservative, theoretical maximum exposure estimate, the U.S. EPA determines how much of the established Reference Dose (RfD) would be utilized by all currently approved product uses. The chronic RfD (cRfD) is an estimate of the amount of daily pesticide exposure to the human population that can occur over a lifetime with a reasonable certainty of no harm to human health[42] . For glyphosate, the chronic RfD is 1.75 mg per kg body weight per day (mg/kg bw/day) (U.S. EPA, 2006a; 2006b). The U.S. EPA will utilize the Chronic Population Adjusted Dose (cPAD, the cRfD with any FQPA uncertainty factors applied) from aggregated exposures and from the exposure assessment to determine if these exposures exceed the U.S. EPA level of concern (i.e., 100 percent of the cPAD).

If the aggregate risk assessment shows that utilization of the cPAD does not exceed the U.S. EPA level of concern, then the U.S. EPA will conclude that the new use does not pose an unreasonable risk to human health. The U.S. EPA will then establish or revise, as needed, any food or animal feed crop tolerances to allow for the presence of glyphosate residue on that crop. However, under a recently announced policy aimed at increasing transparency to the general and regulated public, the U.S. EPA may choose to publish the risk assessment and proposed regulatory decision on the Office of Pesticides website and ask for public comment; this posting occurs prior to the establishment of the tolerances (http://www.epa.gov/pesticides/regulating/registration-status.html). The U.S. EPA publishes the new tolerances in the Federal Register, along with a final summary of the risk assessment and approves pesticide labeling for the new use. In issuing the final tolerance rule, the U.S. EPA considers and discusses any comments received in response to the original notice regarding the U.S. EPA's intention to establish tolerances that was published in the Federal Register and any comments received in the transparency policy notice.

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[42]RfD is the current terminology used by EPA; however earlier EPA risk assessment terminology used the term Allowable Daily Intake (ADI). RfD and ADI are synonymous.

Despite the large number of approved food and feed uses of glyphosate, including uses associated with glyphosate-tolerant crops, a large margin of safety exists for glyphosate. While use of glyphosate has increased in the decade since the introduction of glyphosate-tolerant crops, the associated risk to human health as a result of the increased human exposure to glyphosate remains low, due to the low mammalian toxicity of glyphosate and the relatively low dietary exposure associated with the herbicide's approved uses.

Prior to the first approval of a glyphosate-tolerant crop (soybean) in 1996, theoretical maximum dietary exposure for all registered conventional uses of glyphosate utilized approximately 2.9% of the glyphosate RfD for the most highly exposed subpopulation of non-nursing infants less than one year old (U.S. EPA, 1993a). A more recent U.S. EPA risk estimate determined chronic dietary exposure estimates for food and drinking water to glyphosate to be below the U.S. EPA level of concern for both the general U.S. population and population subgroups; 2% and 9% of the chronic population adjusted dose (cPAD) for the general population and non-nursing infants (most exposed subpopulation) respectively (U.S. EPA, 2006b). The combined short/intermediate-term estimated exposure for dietary and non-dietary exposures with all current registered uses of glyphosate utilizes only 11% of the glyphosate cPAD for the most sensitive subpopulation of non-nursing infants less than one year old (U.S. EPA, 2006b). The utilization of the glyphosate cPAD, which is well below 100 percent, has allowed the U.S. EPA to continue to make the conclusion of reasonable certainty of no harm to human health for each glyphosate use, including new glyphosate-tolerant crop uses.

These figures are supported by the data provided in the tables below. Table K-1 summarizes the established food and feed tolerances supporting the use of glyphosate in the conventional crops of alfalfa, cotton, sugar beet and soybean prior to the first glyphosate-tolerant crop in 1996. A summary of the regulatory approvals, including new or modified food and feed tolerances, and associated dietary exposure assessments for approved glyphosate-tolerant crops is provided in Table K-2. Table K-3 summarizes the most recent chronic and short/intermediate-term aggregate risk assessments for glyphosate.

Table K-1. Established Glyphosate Tolerances in Selected Crops Prior to Glyphosate-tolerant Crops (1993)

Crop	Established Food/Feed Tolerances	Publication	% of Reference Dose (RfD)[1]
Soybean	• Seed – 20 ppm • forage & hay – 15 ppm • hulls – 100 ppm	Glyphosate Re-registration Eligibility Decision Document Sept. 1993 (U.S. EPA, 1993a)	General Population - 1.2 Non-nursing infants <1 year old - 2.9
Alfalfa	200 ppm		
Cotton	forage, hay, & seed – 15 ppm		
Sugar beet	Roots – 0.2 ppm		
Maize	0.1 ppm seed 0.2 ppm forage/fodder		
Canola	none		

[1] % of Reference Dose based on all established tolerances in 1993.

Table K-2. Summary of EPA Approvals for Glyphosate Use in Glyphosate-tolerant Crops

	Commercial Introduction Year	Required Changes in Food/Feed Tolerances	Federal Register Publication Establishing New or Modified Tolerance	Dietary Exposure Only (Food + Water)
Roundup Ready soybean	1996	• Increase soybean forage to 100 ppm. • Increase soybean hay to 200 ppm. • Establish new tolerance for aspirated grain fractions at 50 ppm.	61 FR 15192 Petition No. 4F4369 Apr. 1996 (U.S. EPA, 1996b)	General Population – 1% of RfD Non-nursing infants <1 year old- 2.5% of RfD Children 1-6 yrs – 2.5% of RfD
Roundup Ready cotton	1997	Establish new tolerance for gin byproduct at 100 ppm.	61 FR 7729 Petition No. 5F4493 Feb. 1996 (U.S. EPA, 1996a)	General Population – 1% of RfD Non-nursing infants <1 year old - 2.4% of RfD Children 1-6 yrs – 2.3% of RfD
Roundup Ready corn	1998	Establish new tolerance for maize • forage at 1 ppm • grain at 1 ppm • stover at 100 ppm.	62 FR 17723 Petition No. 5F4555 Apr. 1997 (U.S. EPA, 1997)	General Population – 1% of RfD Non-nursing infants < 1 year old – 3% of RfD Children 1-6 yrs – 3% of RfD
		Establish new tolerance for maize forage at 3 ppm	Sept 27 2000 65 FR 57957 Sep. 2000 U.S. EPA, 2000 #53040}	General Population - 1.5% of RfD All infants <1 year old – 3.1% of RfD Children 1-6 yrs – 3.2% of RfD
Roundup Ready corn 2	2004	Increased tolerance for maize forage to 6 ppm.	68 FR 36472 Jun. 2003 (U.S. EPA, 2003)	Change in forage tolerance did not affect estimated dietary exposure from animal products; therefore no dietary risk assessment was conducted.
Roundup Ready canola	1999	Establish new tolerances for canola. • seed at 10 ppm • meal at 15 ppm	64 FR 18360 Petition No. 2E4118 Apr. 1999 (U.S. EPA, 1999)	General Population - 1.5% of RfD Non-nursing infants <1 year old - 3.3 % of RfD Children 1-6 yrs – 3.2% of RfD
Roundup Ready sugar beet	2008	Establish new tolerances for sugar beet. • roots at 10 ppm • tops at 10 ppm • pulp (dried) at 25 ppm		

Table K-2. Summary of EPA Approvals for Glyphosate Use in Glyphosate-tolerant Crops (Continued)

	Commercial Introduction Year	Required Changes in Food/Feed Tolerances	Federal Register Publication Establishing New or Modified Tolerance	Dietary Exposure Only (Food + Water)
Roundup Ready Flex cotton	2006	• Increase tolerance for gin byproducts to 175 ppm. • Increase tolerance for undelinted cottonseed to 35 ppm.	69 FR 65081 Petition No. 3F6570 Nov. 2004 (U.S. EPA, 2004c)	General Population - 2.2% of cPAD All infants < 1 year old - -3.9% of cPAD Children 1-2 years - 5.4% of cPAD
Roundup Ready alfalfa	2006	Establish new tolerances for alfalfa seed at 0.5 ppm.	70 FR 7861 Petition No. 2F6487 Feb. 2005 (U.S. EPA, 2005)	Dietary exposure insignificant, did not conduct new risk assessment. Deferred to assessment conducted for flex cotton as published in 69 FR 65081.
Maize		Increased tolerance for maize grain to 5 ppm.	73 FR 52607 Sept. 2008 (U.S. EPA, 2008a)	Tolerance adjusted to harmonize with CODEX. Based on conventional and glyphosate-tolerant uses in maize

Table K-3. Aggregate Exposure Assessment for Glyphosate

Population Subgroup	Acute Aggregate[2]	RfD (mg/kg/day) [2]	Chronic Aggregate[1,2]		Short/Intermediate Term Aggregate[2,3]	
			Exposure (mg/kg/day)	% cPAD	Exposure (mg/kg/day)	% RfD
General U.S. population	Not applicable	1.75	0.041	2	-	-
All infants (<1 year)			0.127	7	0.157	9
Non-nursing infants (<1 year)			0.158	9	0.188	11
Children 1-2 years			0.095	5	0.125	7
Children 3-5 years			0.088	5	0.118	7
Children 6-12 years			0.059	3	0.089	5
Youth 13-19 years			0.037	2	-	-
Adults 20-49 years			0.033	2	0.063	4
Adults 50+ years			0.028	2	-	-
Females 13-49 years			0.031	2	-	-

[1]These aggregate exposure assessments were performed by the U.S. EPA prior to the issuance of latest guidance (http://www.epa.gov/pesticides/health/revisedRAmethods.pdf, December 2009), and thus do not include occupational exposure. As such, the chronic aggregate exposure estimated in this fashion was the same as chronic dietary exposure because chronic non-dietary exposure was not expected based upon the registered non-crop uses of glyphosate.

[2]U.S.EPA (2006b).

3Calculated from values given in U.S.EPA (2006b).

K.2.2. Glyphosate Safety Evaluation for Applicator and Bystander Exposure

Another potential impact of the use of glyphosate on human health that the U.S. EPA considers in its human health analysis is applicator and bystander exposure resulting from increased glyphosate use. Based on the toxicity of glyphosate and its registered uses, including use on glyphosate-tolerant crops, the U.S. EPA has concluded that occupational exposures (short-term dermal and inhalation) to glyphosate are not of concern because no short-term dermal or inhalation toxicity endpoints have been identified for glyphosate (U.S. EPA, 2006a; 2006b).

Additional evidence to support the U.S. EPA conclusion can be found in the Farm Family Exposure Study (Acquavella et al., 2004), a biomonitoring study of pesticide applicators conducted by independent investigators. This biomonitoring study determined that the highest estimated systemic dose of glyphosate for applicators as the result of routine labeled applications of registered glyphosate-based agricultural herbicides to crops, including glyphosate-tolerant crops, was approximately 400 times lower than the RfD established for glyphosate. Furthermore, investigators determined that 40% of applicators did not have detectable exposure on the day of application, and 90% of the applicators had an estimated systemic dose of glyphosate less than 0.06% the RfD (Acquavella et al., 2004).

The biomonitoring study also found little evidence of detectable exposure to individuals on the farm who were not actively involved in or located in the immediate vicinity of labeled applications of glyphosate-based agricultural herbicides to crops. Considering the similarity of the use pattern and application rates of the glyphosate products in this study compared to those registered for use on glyphosate-tolerant crops, bystander exposure attributed to the use of glyphosate on glyphosate-tolerant crops is expected to be negligible.

K.3. Potential Impact of Glyphosate on the Environment

Potential environmental effects are carefully considered as a part of the FIFRA pesticide registration process. Prior to the approval of a new pesticide or a new use (including a change in pesticide application rates and/or timing) and before re-registering an existing pesticide, the U.S. EPA must consider the potential for environmental effects and make a determination that no unreasonable adverse effects to the environment will be caused by the new pesticide, new use or continued use.

To make this determination, the EPA requires a comprehensive set of environmental fate and ecotoxicology data on the pesticide's active ingredient (40 CFR § 158). The U.S. EPA uses these data to assess the pesticide's potential environmental risk (exposure/hazard). The required data include both short and long-term hazard data on representative organisms that are used to predict hazards to terrestrial animals (birds, nontarget insects, and mammals), aquatic animals (freshwater fish and invertebrates, estuarine and marine organisms) and nontarget plants (terrestrial and aquatic).

The U.S. EPA re-evaluated the environmental safety of glyphosate in 1993 as part of the FIFRA-required re-registration of all pesticides. At the end of this evaluation, the EPA concluded that all registered uses of glyphosate were eligible for re-registration, including terrestrial (i.e., land-based) applications up to 6 lbs glyphosate acid equivalents (a.e.) per acre on crops, and 8 lbs glyphosate a.e per acre for certain limited uses.

Since the re-registration evaluation in 1993, the U.S. EPA has reviewed and approved a significant number of new glyphosate uses: on conventional crops such as legume vegetables and sunflower/safflower seed, glyphosate-tolerant crops such as alfalfa, maize, cotton, canola, sugar beet and soybean and in non-crop areas. In each case, the EPA concluded that the new use, including any incremental environmental exposure to glyphosate caused by that new use, did not pose an unreasonable risk to the environment, and approved pesticide labeling for the new use.

The studies and data collected by Monsanto, both for the initial U.S. EPA registration and re-registration of glyphosate, as well as data developed by independent academics, present a well-established safety profile for glyphosate. The following sections provide greater detail regarding some of the key findings from these studies.

K.3.1. Persistence of Glyphosate in the Soil

Persistence of agricultural chemicals in the soil is widely regarded as an undesirable environmental characteristic. Glyphosate has been shown to degrade over time from most agricultural ecosystems across a wide range of soil and climatic conditions, with a median soil half-life (the time it takes for half of the glyphosate to dissipate in the soil) of 13.9 days (U.S. EPA, 1993a). The potential for glyphosate to accumulate in soil following repeated applications has been studied both in the laboratory and the field.

A laboratory study was conducted on two soil samples, with each sample receiving up to three sequential applications of 5 pounds glyphosate a.e. per acre over a 6-week period, at two-week intervals. The concentration of glyphosate in soil 24 weeks following application had declined to 1-5% of the concentration immediately after application, regardless of whether it was the first, second or third application.

Glyphosate degradation in the soil following multiple glyphosate applications was also shown under field conditions. Soil was collected from pesticide efficacy and tolerance trials in orchards and vineyards that received repeated applications of glyphosate over a one- to six-year period, at cumulative rates of 6 to 120 pounds glyphosate a.e. per acre. These soil samples did not show any accumulation of glyphosate residues, even at the exaggerated rate of three sequential applications of eight pounds glyphosate a.e. per acre within a three-month interval for five out of six sequential years. Glyphosate degradation continued after multiple applications, and less than 10 percent of the total applied glyphosate remained in the soil one year after the last glyphosate application.

Similar to some of the other glyphosate tolerant crops, a typical agronomic (annual) use pattern for glyphosate on MON 88302 could include a pre-emergence burn down application of up to 3.7 pounds of glyphosate acid equivalent (a.e.) per acre. This could

be followed by one to two post-emergence in-crop applications for weed control of up to a total of 1.6 pounds glyphosate a.e. per acre. The total amount of glyphosate that could be applied per season (pre-emergence through first flower) cannot exceed 5.3 pounds glyphosate a.e. per acre. Thus, the maximum labeled rates and typical use patterns of glyphosate on MON 88302 are well within the rates and frequencies used in the soil persistence studies described in this section. As a result, glyphosate is not expected to accumulate in soil when MON 88302 is applied according to label directions.

K.3.2. Persistence of Surfactant in the Soil

Herbicide products approved for application to emerged weeds normally are applied with surfactants. Glyphosate products are formulated with surfactants to increase the permeability of the cuticle wax of the weed foliage, resulting in increased foliar uptake of glyphosate. In other words, the surfactant acts to alter the physical properties of the plant's natural protective wax coating, allowing the plant to better absorb the glyphosate, thereby improving the efficacy of the herbicide.

One common surfactant used in formulated glyphosate products is polyethoxylated alkyl amine (POEA). When degradation of POEA was investigated in three types of soil (silt loam, silty clay loam and sandy loam), microbial degradation was determined to be the primary degradation route, with minimal degradation occurring under sterile conditions. Approximately 25-30% of applied ^{14}C-POEA was mineralized to $^{14}CO_2$ within seven weeks. The estimated degradation half-life for parent POEA was less than one week and possibly as short as one to two days. Because limited data are available for POEA dissipation, a conservative estimate of half-life values for POEA in soil would be 7-14 days (Giesy et al., 2000). Glyphosate and the POEA surfactant have similar soil dissipation rates and the same primary route of dissipation, i.e., microbial degradation. Therefore, it is reasonable to assume that the POEA surfactant will behave similarly to glyphosate in field soil, and an increase in residual soil concentrations (accumulation) of the POEA surfactant is not anticipated as a result of increased use of glyphosate associated with the planting of MON 88302.

K.3.3. Surface Water and Groundwater

Glyphosate binds strongly to agricultural soils and has a low potential to move offsite to surface water or leach to groundwater (U.S. EPA, 1993a). The U.S. EPA has used computer models to estimate worst-case glyphosate levels in surface water based on presently approved use patterns. Relying on toxicological data from acute and chronic tests on fish and other aquatic organisms, the EPA has determined that "the potential for environmental effects of glyphosate in surface water is minimal" (U.S. EPA, 2002).

K.3.4. Wildlife

Animals

As a part of the re-registration evaluation under FIFRA, the U.S. EPA conducted an ecological assessment for glyphosate. This assessment compared the results from toxicity tests using glyphosate, conducted with various plant and animal species to a conservative estimate of the concentration of glyphosate to which an organism might be exposed in the environment. This estimate, called the Estimated Exposure Concentration (EEC), is a point estimate for exposure that does not take into account normal environmental dilution or dissipation, or the frequency of exposure to the pesticide by wildlife. In the Re-registration Eligibility Decision (RED) for glyphosate (U.S. EPA, 1993a), the exposure estimates were determined assuming an application rate of 5.0625 lb a.e.[43] per acre, which exceeds the maximum labeled use rate for a single application for agricultural purposes. When the EECs were calculated for aquatic plants and animals, the direct application of this rate to water was assumed. Based on this assessment, the U.S. EPA concluded that effects to birds, mammals, fish and invertebrates are minimal based on available data (U.S. EPA, 1993a).

Glyphosate is classified as practically non-toxic to honey bees (which are used to assess effects on nontarget insects in general) and as practically non-toxic to slightly toxic to birds, freshwater fish, marine and estuarine species, aquatic invertebrates and mammals (U.S. EPA, 1993a). Glyphosate has a low octanol-water coefficient, indicating that it has a tendency to remain in the water phase rather than move from the water phase into fatty substances; therefore, it is not expected to accumulate in fish or other animal tissues.

The glyphosate end-use products used in agriculture contain a surfactant to facilitate the uptake of glyphosate into the plant (Ashton and Crafts, 1981). Depending on the surfactant used, the toxicity of the end-use product may range from practically nontoxic to moderately toxic to fish and aquatic invertebrates (U.S. EPA, 1993a). For this reason, the 1993 Glyphosate RED stated that some formulated end-use products of glyphosate needed to be labeled as "Toxic to fish" since some glyphosate products are applied directly to aquatic environments. Due to the associated hazard to fish and other aquatic organisms, glyphosate end-use products that are labeled for applications to aquatic environments generally do not contain surfactant, or contain a surfactant approved for direct application to aquatic environments.

Plants

Glyphosate is a non-selective herbicide with post-emergence activity on essentially all annual and perennial plants. As such, exposure to glyphosate could put aquatic and terrestrial nontarget plants as well as threatened or endangered plants at risk (U.S. EPA, 1993a). Nontarget plants may potentially be at risk from applications of glyphosate as a result of spray drift. As discussed earlier, glyphosate binds tightly to agricultural soils

[43]Although the RED refers to the maximum application rate as a.i., it can be determined from the EEC's obtained that the maximum rate in acid equivalents was actually used in the calculation of the EEC's

and has low potential to move offsite dissolved in water. Moreover, glyphosate is not taken up from agricultural soils by plants. Therefore, risks to nontarget plants are only attributed to the spray drift of the pesticide. Pesticide labels include specific risk management measures to manage spray drift, including mandatory requirements for aerial applications.

During the re-registration process in 1993, additional data on terrestrial nontarget plants were requested by the U.S. EPA. These additional data have been utilized in conjunction with an exposure assessment to further understand the potential risk to nontarget and threatened and endangered plants from the use of glyphosate herbicides in agriculture. Using the methodology described in a recent EPA effects determination for glyphosate (U.S. EPA, 2008a), it can be determined that there is minimal risk to terrestrial plants that are not listed as threatened or endangered at the maximum glyphosate single application rate for ground applications (3.75 lbs a.e. glyphosate per acre), and at rates just below the maximum single aerial application rate (1.48 lbs a.e. glyphosate per acre compared to 1.55 lbs a.e. glyphosate per acre). Nonlisted plants, therefore, are unlikely to be significantly impacted by glyphosate applications to MON 88302. The potential impact to threatened and endangered plant species has been further considered in an analysis conducted by Monsanto (Mortensen et al., 2008).

K.3.5. Endangered and Threatened Species

The U.S. EPA Endangered Species Protection Program web site, http://www.epa.gov/espp/, describes the U.S. EPA assessment process for endangered species. The essential elements of that process, generally taken from the web site, are summarized below.

The Endangered Species Act (ESA) was intended to protect and promote the recovery of animals and plants that are in danger of becoming extinct. All federal agencies are required under the ESA to ensure that their regulatory actions, including the U.S. EPA's registration of pesticides in the U.S., are not likely to jeopardize the continued existence of threatened or endangered species ("listed" species) or destroy or adversely modify their critical habitat.

The U.S. EPA's Endangered Species Protection Program (ESPP) which helps promote the recovery of listed species is designed to determine whether pesticide use in a certain geographic area may affect any listed species.

When registering a pesticide or reassessing the potential ecological risks from use of a currently registered pesticide, the U.S. EPA extensively evaluates environmental fate and ecological effects data to determine how a pesticide will move through and break down in the environment. Risks to birds, fish, invertebrates, mammals and plants are routinely assessed and used in U.S. EPA's determinations of whether a pesticide may be licensed for use in the U.S.

The U.S. EPA's core pesticide risk assessment and regulatory processes ensure that protections are in place for all populations of nontarget species. Because endangered

species may need specific protection, the U.S. EPA has developed risk assessment procedures described in the Overview of the Ecological Risk Assessment Process (U.S. EPA, 2004d) to determine whether individuals of a listed species have the potential to be harmed by a pesticide, and if so, what specific protections may be appropriate. The U.S. EPA's conclusion regarding the potential risks a pesticide may pose to a listed species and any designated critical habitat for the species, after conducting a thorough ecological risk assessment, results in an "effects determination."

An evaluation of the effects of glyphosate use on all types of threatened and endangered species was conducted by Monsanto. This evaluation generally followed the procedures described in the Overview of the Ecological Risk Assessment Process (U.S. EPA, 2004d), as summarized in Figure K-1.

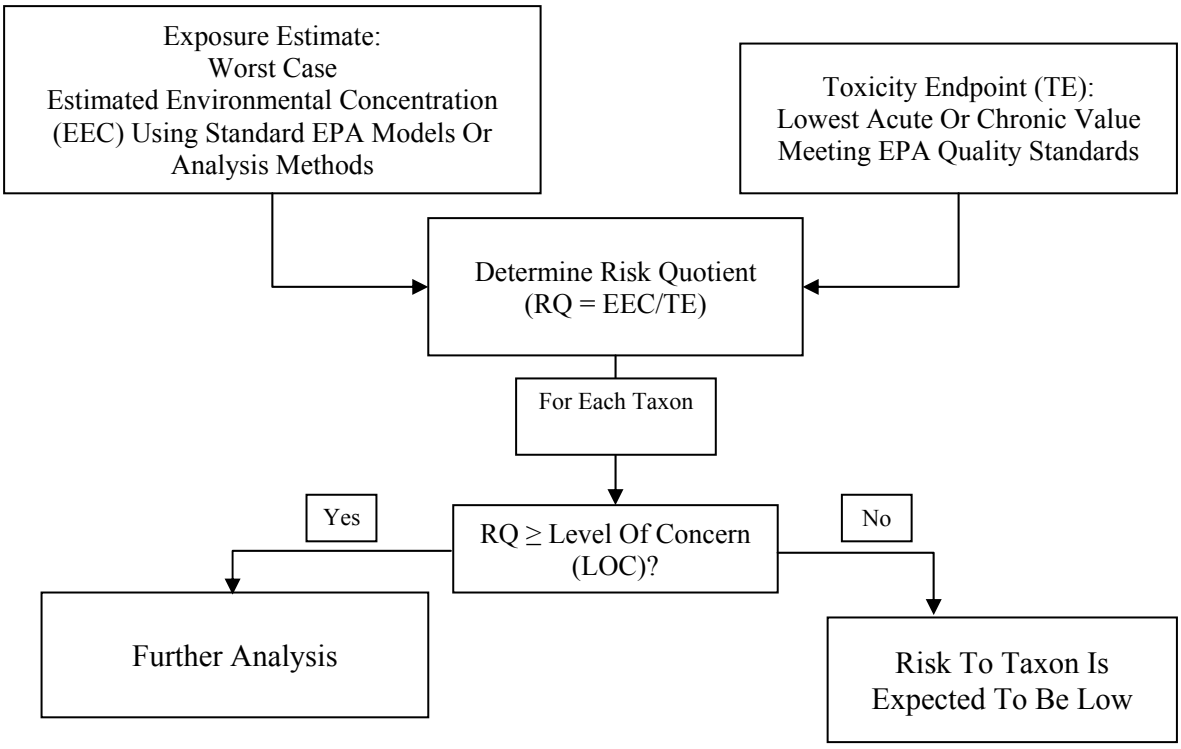

Figure K-1. Tier I Endangered Species Analysis

Risk quotients (RQ's) were calculated as the quotient of the Estimated Environmental Concentration (EEC) and the relevant toxicity endpoint for the most sensitive species for a given taxon (class of species). For acute studies of a few days duration, the concentration calculated to result in 50% mortality (LC_{50}) or 50% designated effect (EC_{50}) on the test species was utilized in the RQ calculation. For chronic studies, representing a significant portion of the species life-cycle, the highest concentration at which no effects were observed (No Observed Effect Concentration, NOEC) was used in the RQ calculation.

Toxicity values (effects endpoints) for most categories of species were taken from a recent U.S. EPA assessment for a new glyphosate use (U.S. EPA, 2006c), or from the U.S. EPA guideline studies conducted by Monsanto if these endpoints were lower. Studies from the literature were considered when the study design was appropriate for the assessment being made and where sufficient information regarding glyphosate or formulation test concentrations was available. Exposure estimates were based on standard U.S. EPA methods for calculating exposure (U.S. EPA, 2004d). For aquatic organisms, the model GENEEC2 (U.S. EPA, 2004d), which calculates high-end estimates of surface water concentrations of pesticides in a generic farm pond, was utilized. When formulation toxicity was considered, default drift values and the U.S. EPA standard pond[44] were utilized for estimation of aquatic exposure. For terrestrial

[44]A water body with a depth of 2 m and a volume of 20,000 liters.

animals, the T-REX model (U.S. EPA, 2008c) was utilized to calculate estimated dietary exposure and risk. For terrestrial and semi-aquatic plants, only the drift component of the Terrplant model (U.S. EPA, 2004d) was used to determine exposure levels (the runoff component was disregarded). Runoff was not considered to contribute to exposure, since glyphosate binds very tightly to agricultural soils and does not have herbicidal properties when bound to soil (U.S. EPA, 2006c).

Risk quotients calculated using toxicity endpoints and estimated exposure concentrations determined as described above were compared with the U.S. EPA established Levels of Concern (U.S. EPA, 2004d). If the RQ exceeded the Endangered Species Level of Concern, a conclusion was reached that a threatened or endangered species may potentially be affected by the glyphosate use. Because initial RQ calculations are made with worst case exposure estimates, further refinement of the estimated exposure concentration or of toxicity endpoints may subsequently indicate the RQ is not as high as initially calculated. If the RQ is less than the Endangered Species Level of Concern it can be concluded that the species will not be affected by glyphosate use.

The conclusion from this evaluation, submitted to the USDA and the U.S. EPA, is that threatened or endangered terrestrial or semi-aquatic plant species are not at risk[45] from ground applications of glyphosate at rates less than 3.5 lb glyphosate a.e. per acre, or from aerial applications at rates less than 0.70 lb a.e. per acre. However, potential effects to these species cannot be excluded when rates exceed these levels. All in-crop applications to MON 88302 will be 1.6 lb a.e. per acre or less, which are not predicted to affect threatened and endangered plants if applications are made using ground equipment. Rates that exceed 3.5 lb a.e. per acre, if used by growers, are generally for control of perennial species prior to crop emergence or prior to harvest.

The same evaluation determined that other taxa (including birds, mammals, insects, fish, amphibians, aquatic invertebrates and non-vascular aquatic plants) would not be affected by the use of glyphosate herbicides in crop production. Furthermore, this evaluation determined that these other taxa were not at risk from indirect effects resulting from habitat alteration from the use of glyphosate, since non-endangered terrestrial or semi-aquatic plants were not considered to be at risk of direct effects.

Based on Monsanto's determination that threatened and endangered plant species potentially could be affected by certain uses of glyphosate in crop production (e.g., aerial applications at the maximum aerial rate), a more detailed evaluation of the locations of threatened and endangered plant species relative to areas of crop production has been undertaken. The first crop to be assessed was alfalfa (Honegger et al., 2008), but canola, maize, cotton, soybeans and sugar beets have now also been evaluated. The evaluation process was divided into three phases, as outlined below.

- First, the co-occurrence of observations of threatened or endangered plant species and the presence of alfalfa, canola, maize, cotton, soybeans or sugar beet

[45] Risk to threatened or endangered plant species is only assessed outside of agricultural production areas.

production was determined at the county level. This analysis (Phase 1) considered the 3028 counties in which at least one of these six crops are grown, which comprise 96% of the 3141 counties and equivalent areas[46] in the 50 states of the U.S. Species were reviewed for applicable exclusions at the county-level, which indicated, for some species, that glyphosate use in these crops posed no risk of adverse effects to these species.

- Next, for threatened or endangered plant species where effects could not be excluded at the county level, the possible exposure to glyphosate was assessed at the sub-county level (Phase 2) in the same counties considered in Phase 1. This analysis used information available at the sub-county level for threatened and endangered plant species locations and for land use. Land uses considered in this analysis are those classified as Pasture/Hay and Cultivated Crops.[47]
- Finally, in sub-county areas where, under certain application conditions, the potential for threatened and endangered plant species to be affected by exposure to glyphosate could not be excluded, areas have been defined so that grower practices can be implemented to limit glyphosate exposure (Phase 3). Measures to limit glyphosate exposure in these areas have been proposed. These measures include: 1) limiting ground application rates to less than 3.5 lb glyphosate a.e. per acre in areas identified for potential use limitation when the potential habitat for the threatened or endangered species is present and 2) for aerial applications greater than 0.7 lb a.e./acre, implementing an unsprayed buffer between the potential habitat for the listed species and the application area. Proposed buffer distances are based on application rate, droplet size and wind direction.

This analysis was initially completed for the U.S. counties in which alfalfa, canola, maize, cotton, soybeans and sugar beets were grown based on the 2002 Ag Census (USDA-NASS, 2002) and listed species information available through early 2008.

Of the 3028 U.S. counties where alfalfa, canola, maize, cotton, soybeans and sugar beets were produced, 11% of counties (334 counties) required the definition of potential areas for use limitations. In the other 2694 counties, either there were no threatened or endangered plant species present, or the species present were either excluded from concern (based on habitat or proximity information), had existing protections, or were not in proximity to potential areas of production of the six crops evaluated. This analysis is being updated based on the 2007 Ag Census (USDA-NASS, 2009) and updated listed species information.

The Roundup Ready crop analysis considered the land classifications where agricultural crop production may occur (in counties with reported farms producing any of the six crops) in the assessment of proximity to observations of threatened or endangered

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[46] Equivalent areas include independent cities that are not within the boundaries of a county.

[47] Land use was based on the National Land Cover Database (2001) for the continental U.S. and on the NOAA (National Oceanic and Atmospheric Administration) Coastal Services Center land cover data for Hawaii.

species. Thus, the identification of potential use limitation areas also applies to other crops in those counties.

K.3.6. Potential Effects on Endangered Animal Species Identified by the U.S. EPA or in Litigation

As previously discussed, no indirect effects on threatened or endangered animal species are predicted, since no significant direct effects due to pesticide drift onto non-endangered plant species are predicted. In the Glyphosate RED (U.S. EPA, 1993a), the U.S. EPA suggested that glyphosate may have effects on the habitat of the Houston Toad. After the issuance of the 1993 Glyphosate RED, Monsanto conducted a vegetative vigor study. When relevant effects data from that study are considered, it can be determined that the amount of glyphosate per unit area predicted to drift away from the site of an agricultural application is less than the amount per unit area observed to have a 25% effect on plant dry weight or growth of the most sensitive of ten species tested in the study. Thus, the habitat of the toad is not likely to be significantly affected by glyphosate drift, and hence the toad is not likely to be at risk from the agricultural use of glyphosate.

The EPA evaluated the effect of glyphosate on the California Red-legged Frog in response to a consent agreement reached in a lawsuit filed by the Center for Biological Diversity[48] . In the California red-legged frog effects determination (U.S. EPA, 2008b), the U.S. EPA considered glyphosate rates up to 7.95 lb a.e. per acre. Even at this high rate, the U.S. EPA concluded that there would be no direct effect to the aquatic phase of the California red-legged frog (CRLF) from application of glyphosate or any glyphosate formulation or salts[49] . The U.S. EPA also concluded that there are no direct effects to the terrestrial-phase of the CRLF at rates of 3.85 lb a.e. per acre and below (with the exception of one formulation that is not registered for use in MON 88302). [50] Since the maximum in-crop application rate for MON 88302 is 1.6 lb a.e. per acre, glyphosate formulations and rates used in MON 88302 do not pose any potential risk of direct effects to the aquatic or terrestrial-phase of the CRLF.

With respect to indirect effects on prey, the U.S. EPA concluded that no effect on the following prey items: algae, aquatic invertebrates, aquatic-phase frogs or fish, or terrestrial-phase frogs would occur at glyphosate rates of 3.84 lb a.e. per acre.[51] The potential for some effects on terrestrial invertebrate and small mammal prey items were identified but only at rates above 7.5 lb a.e. per acre and 3.75 lb a.e. per acre, respectively.[52] No chronic effects on mammals were predicted at rates of 3.75 lb a.e. per

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[48] ***Center For Biological Diversity v. Leavitt*****,** 2005 WL 2277030 (N.D.Cal., September 19, 2005).

[49](U.S. EPA, 2008a)Page 11, "The acute and chronic LOC's for freshwater fish and aquatic-phase amphibians are not exceeded for either glyphosate, its salts or its formulations".

[50]Ibid., Page 14, Table 1.3.

[51]Ibid., Page 15, Table 1.4.

[52]Ibid., Page 156, ibid. For terrestrial invertebrate prey items that were not listed (i.e. not threatened or endangered), no effects were predicted at any rate up to 7.95 lb a.e. per acre for large invertebrates, and the Level of Concern was only exceeded for small invertebrates at application rates of 7.5 lb a.e. per acre and above. Levels of Concern were exceeded at lower rates for listed (threatened or endangered) terrestrial invertebrates, but listed species would not be anticipated to be a significant portion of the CRLF diet. Listed small invertebrates in areas adjacent to a MON 88302 field, would not be at risk from spray drift exposure from glyphosate applications to MON 88302.

acre and below.[53] Therefore, no effects on CRLF prey were identified at the maximum single application rate for MON 88302. Similarly, no effects on terrestrial plant habitat are identified for ground applications at 1.6 lb a.e. per acre. [54]

Based on the CRLF effects determination conducted by the U.S. EPA, it is not expected that the glyphosate rates applied to MON 88302 would have any direct or indirect impact on the CRLF.

The EPA also has evaluated the potential effect of glyphosate on salmon in eleven areas in California and Southern Oregon[55] in response to the consent agreement reached in another lawsuit[56]. The conclusion of the U.S. EPA's risk assessment was that for all uses of glyphosate with application rates of 5 lb active ingredient (a.i.) per acre or below, the Agency determined that glyphosate will have no effect on the subject listed species. (U.S. EPA, 2004a; 2004b). All glyphosate use rates for agricultural uses are 5 lb a.i. per acre (3.7 lb glyphosate a.e. per acre) or below, so no risk to salmon is anticipated from these uses.

K.3.7. Other Potential Environmental Impacts Associated with Glyphosate Use in Glyphosate-tolerant Crops

As discussed more fully below, the potential impacts to soil attributable to the change in production (cultivation) practices associated with the deregulation of glyphosate-tolerant crops have been assessed. The adoption of glyphosate-tolerant crops and the ability to use glyphosate-based agricultural herbicides is not expected to significantly change agricultural practices, except to enable the adoption of no-till seeding practices.

No-Till Practices: No-till production is the practice of establishing an agricultural seed bed and controlling weeds without mechanically tilling the soil. Instead, the only tillage of the soil is done at the time of planting, with the crop being seeded directly into the previous year's crop residue. Among other environmental benefits, no-till production reduces soil erosion and the use of petroleum-based fuels for tractors. The practice has been shown to minimize surface water runoff and soil erosion and to improve soil quality by increasing the soil organic matter that helps bind soil nutrients and prevent their loss to runoff, erosion and leaching (Leep et al., 2003).

No-till agriculture can provide benefits to water bodies, as well. No-till practices reduce soil erosion to surface water bodies, decreasing the amount of sediment in rivers and

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[53]Ibid., Page 12. The potential for chronic effects on small mammals were only identified at rates of 3.84 lb a.e. and above (Risk Quotient (RQ) greater than the LOC). Since the next lower rate considered in the CRLF evaluation is 3.75 lb a.e.per acre, it can be concluded that the RQ did not exceed the LOC for these rates, and, therefore, no effects would be anticipated from an application rate of 1.6 lb a.e.per acre)

[54]Ibid., Page 136-138. Risk quotients < 1 are below the Level of Concern. Table 5.6 and 5.7 indicate RQ's for ground applications of 1.54 lb a.e.per acre are well below the LOC of 1. Since the maximum in-crop application rate for MON 88302 is 1.6 lb a.e.per acre no risk of indirect effects to CRLF because of effects on plants in the habitat are predicted from glyphosate use on MON 88302.

[55] These areas are call Evolutionarily Significant Units based on the salmonid populations present in these areas.

[56] *Washington Toxics Coalition v. Environmental Protection Agency*, 413 F.3d 1024 (9th Cir. 2005).

streams. Sedimentation increases the turbidity (cloudiness) of surface water bodies, reducing light penetration, impairing photosynthesis and altering oxygen levels, which cause a reduction of food sources for some aquatic organisms. Sediment can also cover spawning beds and impact fish populations. Phosphorus (a major component of fertilizer) bound to soil particles can be transferred to rivers and lakes via soil erosion, giving rise to high levels of phosphorus in surface waters, which may lead to algae blooms that can impact desirable fish populations (Hill and Mannering, 1995).

Soil Microorganisms: Results of standardized tests with glyphosate formulations performed for submission to regulatory agencies indicate no long-term effects on microorganisms in soil even at rates that exceed maximum use rates (up to five times the labeled rate). In addition, independent researchers have reviewed numerous laboratory and field studies, investigating the effects of glyphosate on soil bacteria and fungi (Felsot, 2000; Giesy et al., 2000). Although some laboratory tests have shown effects on nitrogen-fixing bacteria (Moorman et al., 1992; Santos and Flores, 1995) and soil fungi (Busse et al., 2001; Estok et al., 1989), effects are typically observed only under artificial laboratory conditions and at glyphosate concentrations well above normal field application rates. Several researchers have concluded that it is difficult to extrapolate results from the laboratory to the natural soil environment (Busse et al., 2001; Estok et al., 1989; Wan et al., 1998; Wardle and Parkinson, 1990b).

In studying microorganisms from soil in pine plantations, (Busse et al., 2001) noted: "Our findings suggest that artificial media assays are of limited relevance in predicting glyphosate toxicity to soil organisms and that field rate applications of glyphosate should have little or no effect on soil microbial communities in ponderosa pine plantations." Long-term studies following repeated applications of Roundup agricultural herbicides in the field at labeled use rates for multiple applications in one year (Olson and Lindwall, 1991) or over 15 years (Biederbeck et al., 1997; Hart and Brookes, 1996) have shown no long-term adverse effects on soil microbes. Investigations by Haney et al. . (2002; 2000) related to the increased use of glyphosate-tolerant crops indicate that glyphosate was degraded over time by soil microbes, even at high application rates, without adversely impacting the soil microbial community. In addition, results from field studies that have evaluated the fungal component of the soil microbial community indicate that glyphosate treatment had no deleterious effects on beneficial soil fungi (Araujo et al., 2003; Biederbeck et al., 1997; Busse et al., 2001; Wardle and Parkinson, 1990a; 1990b). Moreover, the history of safe use and yield data obtained for nearly 10 years of glyphosate-tolerant crop production, combined with in-crop applications of glyphosate-based agricultural herbicides, reinforce the findings that soil microbes and microbially-mediated processes are not adversely impacted by field-rate applications of glyphosate.

The Potential for Glyphosate Metal Chelation to Affect Soil Fertility: Plants are dependent on the uptake of a number of different metal cations from the soil for optimal growth. Glyphosate is known to chelate, or tightly bind, to several di- and trivalent metal cations such as $Fe^{3+,}$ Cu^{2+}, Mn^{2+}, Al^{3+} and Ca^{2+} that are needed by plants (Glass, 1984; Madsen et al., 1978). Cations that chelate glyphosate have been shown to reduce the efficacy of glyphosate when present in sufficient amounts in the tank mix spray solution (Bernards et al., 2005). In the spray solution, there is a simple interaction between

glyphosate and metal cations, which reduces the herbicidal activity of glyphosate. However, in the soil environment, the interactions between metals and chelators are much more complex (Parker et al., 2005). Glyphosate can interact with metals that are present on the surface of soil particles, as well as with dissolved metal ions in the water soil solution. In addition to glyphosate, many other potential ligands or chelators are present in soil that can also interact with metals. As a result, there is a complex multi-component equilibrium between glyphosate, other ligands or chelators, and numerous metals present in soil. Glyphosate is only one factor in this system. Numerous compositional analysis studies have demonstrated a lack of any significant immobilization of mineral nutrients by glyphosate in soil that results in reduced uptake by plants. These studies have shown that glyphosate-tolerant crops that have been sprayed with glyphosate do not have decreased micronutrient levels compared to untreated controls (McCann et al., 2006; Obert et al., 2004; Ridley et al., 2002).

Transport through the Soil – Surfactant: Available data also suggest that the POEA surfactant used in Roundup agricultural herbicides binds strongly to soil (estimated soil organic carbon-water partition coefficient (K_{oc}) values range from 2500 to 9600[57]) and undergoes microbial degradation with an estimated half-life of less than 14 days (Marvel et al., 1974). POEA is rapidly partitioned (half-life of 13 to 18 hours) from water to sediment in a water / sediment study (Wang et al., 2005). The rapid partitioning of the POEA surfactant to soil / sediment combined with the high K_{oc} values indicates that the surfactant will be tightly bound to the soil. The Groundwater Ubiquity Score (GUS) is an index that indicates the potential for compounds to leach from soil into groundwater based on their half-life and K_{oc} (Gustafson, 1989). Using an estimated half-life of 14 days and a K_{oc} of 2500 as conservative estimates of the rate of degradation and binding to soil, the GUS index for the POEA surfactant is 0.69. According to the GUS movement ranking, this GUS index indicates that POEA has a very low potential to leach to groundwater.

[57] Estimated from the partition ratio between water and sterile soil as reported in the POEA soil degradation study (Marvel et al., 1974).

Attachment 2. Appendix K. U.S. Glyphosate Tolerances for Food & Feed Commodities (40 CFR § 180.364)

Commodity	Parts/million	Commodity	Parts/million
Acerola	0.2	Durian	0.2
Alfalfa, seed	0.5	Egg	0.05
Almond, hulls	25	Epazote	1.3
Aloe vera	0.5	Feijoa	0.2
Ambarella	0.2	Fig	0.2
Animal feed, nongrass, group 18	400	Fish	0.25
Artichoke, globe	0.2	Flax, meal	8.0
Asparagus	0.5	Flax, seed	4.0
Atemoya	0.2	Fruit, citrus, group 10	0.5
Avocado	0.2	Fruit, pome, group 11	0.2
Bamboo, shoots	0.2	Fruit, stone, group 12	0.2
Banana	0.2	Galangal, roots	0.2
Barley, bran	30	Ginger, white, flower	0.2
Barley, grain	20	Goat, kidney	4.0
Beet, sugar, dried pulp	25	Goat, liver	0.5
Beet, sugar, roots	10	Gourd, buffalo, seed	0.1
Beet, sugar, tops	10	Governor's plum	0.2
Berry group 13	0.2	Gow kee, leaves	0.2
Betelnut	1.0	Grain, aspirated fractions	100
Biriba	0.2	Grain, cereal, forage, fodder and straw, group 16, except maize forage	100
Blimbe	0.2	Grain, cereal, group 15, except barley, commercial maize, grain sorghum, oat and wheat	0.1
Borage, seed	0.1	Grape	0.2
Breadfruit	0.2	Grass, forage, fodder and hay, group 17	300
Cacao bean	0.2	Guava	0.2
Cactus, fruit	0.5	Herbs subgroup 19A	0.2
Cactus, pads	0.5	Hog, kidney	4.0
Canistel	0.2	Hog, liver	0.5
Canola, meal	15	Hop, dried cones	7.0
Canola, seed	10	Horse, kidney	4.0
Cattle, kidney	4.0	Horse, liver	0.5
Cattle, liver	0.5	Ilama	0.2
Chaya	1.0	Imbe	0.2
Cherimoya	0.2	Imbu	0.2
Citrus, dried pulp	1.5	Jackfruit	0.2
Coconut	0.1	Jaboticaba	0.2
Coffee, bean	1.0	Jojoba, seed	0.1
Maize, field, forage	6.0	Juneberry	0.2
Maize, field, grain	1.0	Kava, roots	0.2
Cotton, gin byproducts	175	Kenaf, forage	200
Cotton, undelinted seed	35	Kiwifruit	0.2
Cranberry	0.2	Lesquerella, seed	0.1
Crambe, seed	0.1	Leucaena, forage	200
Custard apple	0.2	Lingonberry	0.2

Commodity	Parts/million	Commodity	Parts/million
Date	0.2	Longan	0.2
Dokudami	2.0	Lychee	0.2
Mamey apple	0.2	Sapote, black	0.2
Mango	0.2	Sapote, mamey	0.2
Mangosteen	0.2	Sapote, white	0.2
Marmaladebox	0.2	Sesame, seed	0.1
Meadowfoam, seed	0.1	Sheep, kidney	4.0
Mioga, flower	0.2	Sheep, liver	0.5
Mustard, seed	0.1	Shellfish	3.0
Noni	0.20	Sorghum, grain, grain	15
Nut, pine	1.0	Soursop	0.2
Nut, tree, group 14	1.0	Soybean, forage	100
Oat, grain	20	Soybean, hay	200
Okra	0.5	Soybean, hulls	100
Olive	0.2	Soybean, seed	20
Oregano, Mexican, leaves	2.0	Spanish lime	0.2
Palm heart	0.2	Spearmint, tops	200
Palm heart, leaves	0.2	Spice subgroup 19B	7.0
Palm, oil	0.1	Star apple	0.2
Papaya	0.2	Starfruit	0.2
Papaya, mountain	0.2	Stevia, dried leaves	1.0
Passionfruit	0.2	Strawberry	0.2
Pawpaw	0.2	Sugar apple	0.2
Pea, dry	8.0	Sugarcane, cane	2.0
Peanut	0.1	Sugarcane, molasses	30
Peanut, hay	0.5	Sunflower	85
Pepper leaf, fresh leaves	0.2	Sunflower, seed	0.1
Peppermint, tops	200	Surinam cherry	0.2
Perilla, tops	1.8	Tamarind	0.2
Persimmon	0.2	Tea, dried	1.0
Pineapple	0.1	Tea, instant	7.0
Pistachio	1.0	Teff, grain	5.0
Pomegranate	0.2	Ti, leaves	0.2
Poultry, meat	0.1	Ti, roots	0.2
Poultry, meat byproducts	1.0	Ugli fruit	0.5
Pulasan	0.2	Vegetable, leafy, brassica, group 5	0.2
Quinoa, grain	5.0	Vegetable, bulb, group 3	0.2
Rambutan	0.2	Vegetable, cucurbit, group 9	0.5
Rapeseed, meal	15	Vegetable, foliage of legume, except soybean, subgroup 7A	0.2
Rapeseed, seed	10	Vegetable, fruiting, group 8	0.1
Rose apple	0.2	Vegetable, leafy, except brassica, group 4	0.2
Safflower	85	Vegetable, leaves of root and tuber, group 2, except sugar beet	0.2
Safflower, seed	0.1	Vegetable, legume, group 6, except soybean	5.0
Salal	0.2	Vegetable, legume, group 6 except soybean and pea,dry	5.0

Commodity	Parts/million	Commodity	Parts/million
Sapodilla	0.2	Vegetable, root and tuber, group 1, except sugar beet	0.2
Wasabi, roots	0.2	Wheat, grain	5.0
Water spinach, tops	0.2	Wheat, middlings	20
Watercress, upland	0.2	Wheat, shorts	20
Wax jambu	0.2	Yacon, tuber	0.2
Wheat, bran	20		

References for Appendix K

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March 27, 2012

Dr. John Turner
United States Department of Agriculture
Biotechnology Regulatory Services, APHIS
4700 River Road, Unit 147
Riverdale, MD 20737-1236

RE: Waiver of Confidential Business Information (CBI) Claim for Petition 11-188-01p (Determination of Non-regulated Status for Glyphosate-Tolerant Canola MON 88302)

Dear Dr. Turner:

Monsanto Company has developed a second-generation glyphosate-tolerant canola product, MON 88302, designed to provide growers with improved weed control through greater flexibility for glyphosate herbicide application. MON 88302 produces the same 5-enolpyruvylshikimate-3-phosphate synthase (CP4 EPSPS) protein that is produced in commercial Roundup Ready® crop products, via the incorporation of a *cp4 epsps* coding sequence. The CP4 EPSPS protein confers tolerance to the herbicide glyphosate, the active ingredient in the family of Roundup agricultural herbicides. Monsanto has requested a determination from APHIS that MON 88302 be granted nonregulated status under 7 CFR Part 340. In support of this request Monsanto submitted petition #11-188-01p on June 27th, 2011 which is currently under review by APHIS.

Monsanto does not object to APHIS publishing for public comment, the un-redacted version of Monsanto's petition for the determination of non-regulated status for MON 88302 that Monsanto submitted to APHIS on June 27th 2011, and APHIS deemed complete on October 3rd 2011. As we explained in our letter and supporting analysis provided to APHIS on February 22nd, 2012, Monsanto's Confidential Business Information (CBI) claim for certain information in our draft petition extends until such time as: 1) APHIS determines the petition to be "complete"; and 2) APHIS makes the final petition available for public comment. Therefore, we hereby waive all prior CBI claims related to this completed petition upon APHIS' publication of the same for public comment.

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Should you have any questions concerning this letter, Petition #11-188-01p, or if you wish to set up a meeting for further discussion, please contact Daniel Jenkins, U.S. Agency Regulatory Affairs Lead, Washington D.C., at 202-383-2851, or myself at 314-694-2680 or at margaret.a.wideman@monsanto.com.

Sincerely,

Margaret A. Wideman

Margaret A. Wideman
Regulatory Affairs Manager

cc: Daniel Jenkins/Monsanto
Regulatory files/11-CA-233U