

CANADIAN FOOD INSPECTION AGENCY  
www.inspection.gc.ca

Français

Home

Contact Us

Help

Search

canada.gc.ca

Site Map

[Plants > Biotechnology / PNTs > Unconfined Release > Decisions](#)**About the CFIA**[Acts and Regulations](#)[Food](#)[Animals](#)[Plants](#)[Plant Biosafety](#)

- Current Issues
- Applicants
- General Public
- Notices of Submission Project
- PNTs: Approved and Under Review
- Contacts

 [E-mail This Page](#)**Proactive Disclosure****Decision Document DD2007- 68****Determination of the Safety of Syngenta Seeds Inc.'s Corn (*Zea mays L.*) Event MIR604**

This Decision Document has been prepared to explain the regulatory decision reached under Directive 94-08 (Dir94-08), entitled "*Assessment Criteria for Determining Environmental Safety of Plants with Novel Traits*", its companion biology document BIO1994-11, "*The Biology of Zea mays L. (Corn/Maize)*", and Directive 95-03 (Dir95-03), entitled "*Guidelines for the Assessment of Novel Feeds: Plant Sources*".

The Canadian Food Inspection Agency (CFIA), specifically the specifically the Plant Biosafety Office, of the Plants Products Directorate, the Biotechnology Environmental Release Assessment Unit of the Science Strategies Directorate and the Feed Section of the Animal Health and Production Division have evaluated information submitted by Syngenta Seeds Inc. This information is in regard to the corn rootworm (*Diabrotica spp.*) resistant corn event MIR604. The CFIA has determined that this plant with novel traits (PNT) does not present altered environmental risk nor, as a novel feed, does it present livestock feed safety concerns when compared to currently commercialized corn varieties in Canada.

**Unconfined release into the environment and use as livestock feed of corn event MIR604 is therefore authorized by Plant Biosafety Office of the Plant Products Directorate and the Feed Section of the Animal Health and Production Division as of July 20, 2007. The authorization is limited to three years. Renewal of the three year authorization is conditional upon the submission of additional research results related to rootworm resistance management. Any corn lines derived from event MIR604 may also be released into the environment and used as livestock feed, provided that (i) no inter-specific crosses are performed, (ii) the intended uses are similar, (iii) it is known based on characterization, that these plants do not display any additional novel traits and are substantially equivalent to currently grown corn varieties in Canada, in terms of their potential environmental impact and livestock feed safety, (iv) the novel genes are expressed at a level similar to that of the authorized line and (v) the insect resistance management requirements described in the present document are applied.**

**Corn event MIR604 is subject to the same phytosanitary import requirements as its unmodified counterpart.**

Please note, that the livestock feed and environmental safety of novel feeds and Inc. are critical steps in the potential commercialization of these plant types. Other requirements, such as the evaluation of food safety by Health Canada, have been addressed separately from this review.

**Table of Contents**[I. Brief Identification of the Modified Plant](#)[II. Background Information](#)[III. Description of the Novel Trait](#)

1. Corn Rootworm Resistance
2. Expression of Phosphomannose Isomerase
3. Development Method

#### 4. Stable Integration into the Plant Genome

#### IV. Criteria for the Environmental Assessment

1. Potential of Corn event MIR604 to Become a Weed of Agriculture or be Invasive of Natural Habitats
2. Potential for Gene Flow from Corn event MIR604 to Wild Relatives Whose Hybrid Offspring May Become More Weedy or More Invasive
3. Altered Plant Pest Potential of Corn event MIR604
4. Potential Impact of Corn event MIR604 on Non-Target Organisms
5. Potential Impact of Corn event MIR604 on Biodiversity
6. Potential for Development of Rootworm Resistance to corn event MIR604

#### V. Criteria for the Livestock Feed Assessment

1. Potential Impact of Corn event MIR604 on Livestock Nutrition
2. Potential Impact of Corn event MIR604 on Livestock and Workers/Bystanders

#### VI. New Information Requirements

#### VII. Regulatory Decision

### **I. Brief Identification of the Modified Plant**

Designation of the Modified Plant:	Corn Event MIR604, OECD Unique Identifier SYN-IR604-5
Applicant:	Syngenta Seeds Canada Inc.
Plant Species:	Maize ( <i>Zea mays</i> L.)
Novel Traits:	Resistance to Western and Northern Corn Rootworms. ( <i>Diabrotica virgifera virgifera</i> and <i>Diabrotica barberi</i> )
Trait Introduction Method:	<i>Agrobacterium</i> -mediated transformation
Proposed Use of the Modified Plant:	Production of corn for human consumption (wet mill products, dry mill products and seed oil) and oil, meal, grain, silage and other byproducts for livestock feed. These plants are not intended to be grown outside the normal production area for corn in Canada.

### **II. Background Information**

Syngenta Seeds Inc. developed, through the use of recombinant DNA techniques, a corn event resistant to corn rootworm (*Diabrotica spp.*), a pest of corn in Canada. The corn event, designated as MIR604, was developed to provide a method to control yield losses from insect feeding damage caused by rootworm larvae.

Corn event MIR604 was developed using recombinant DNA technology, resulting in the introduction of the modified cry3A (*mcry3A*) gene and the *pmi* marker gene. The *mcry3A* gene is derived from the *cry3A* gene from *Bacillus thuringiensis* subsp. *tenebrionis* and encodes a modified  $\delta$ -endotoxin Cry3A protein, with enhanced activity against corn rootworms. The *pmi* gene from *Escherichia coli* encodes the phosphomannose isomerase (PMI) enzyme. Expression of the PMI enzyme allows the plant to utilize mannose as a carbon source. This trait is of no agronomical interest but was used to select transformed from non-transformed plants during the development phase of corn event MIR604.

Syngenta Seeds Inc. has provided data on the identity of corn event MIR604, a detailed description of the transformation method, data and information on the gene insertion site, gene copy number and levels of gene expression in the plant and the role of the inserted genes and regulatory sequences. The novel proteins were identified and characterized. Data was provided for the evaluation of the potential toxicity of the novel proteins to livestock and non-target organisms and potential allergenicity of the novel proteins to humans and to livestock.

Corn event MIR604 has been field tested in the United States and the data for trial years 2002 and 2003 were submitted.

Agronomic characteristics of corn event MIR604 such as seedling emergence, plant height, time to reproduction, lodging, susceptibilities to various corn pests and pathogens, and yield parameters were compared to those of unmodified corn counterparts.

Nutritional components of corn event MIR604, such as proximates, amino acids and fatty acids were compared with those of unmodified corn counterparts.

The Biotechnology Environmental Release Assessment (BERA) Unit of the Science Strategies Directorate, CFIA, has reviewed the above information, in light of the assessment criteria for determining environmental safety of PNTs, as described in the Directive 94-08 (Dir94-08), entitled "*Assessment Criteria for Determining Environmental Safety of Plants With Novel Traits*". The BERA Unit has considered:

- potential of corn event MIR604 to become a weed of agriculture or be invasive of natural habitats;
- potential for gene flow from corn event MIR604 to wild relatives whose hybrid offspring may become more weedy or more invasive;
- potential for corn event MIR604 to become a plant pest;
- potential impact of corn event MIR604 or its gene products on non-target species, including humans; and
- potential impact of corn event MIR604 on biodiversity.

The Feed Section of the Animal Health and Production Division, CFIA, has also reviewed the above information with respect to the assessment criteria for determining the safety and efficacy of livestock feed, as described in Directive 95-03 (Dir95-03), entitled "*Guidelines for the Assessment of Novel Feeds: Plant Sources*". The Feed Section has considered:

- potential impact of corn event MIR604 on livestock nutrition; and
- potential impact of corn event MIR604 on livestock and workers/bystanders

Syngenta Seeds Inc. has provided the CFIA with a method for the detection and identification of corn products containing the corn event MIR604.

### III. Description of the Novel Trait

#### 1. Resistance to Corn Rootworms (*Diabrotica spp.*)

*Bacillus thuringiensis* subsp. *tenebrionis* is a common gram-positive soil-borne bacterium. In the spore forming stage, it produces several insecticidal protein crystals, including the  $\delta$ -endotoxin Cry3A protein which is active against certain coleopteran insects, such as the Colorado potato beetle. The protein is insecticidal to susceptible coleopterans after cleavage by proteases in the insect's gut, forming a protease-resistant active fragment that is the bio-active form of the protein. Insecticidal activity is believed to depend on the binding of the active fragment to specific receptors present in susceptible insects on midgut epithelial cells, forming pores which disrupt osmotic balance and eventually result in cell lysis and insect death. However, the native Cry3A does not have significant activity against corn rootworms due to its insensitivity to corn rootworm protease. Syngenta modified the native cry3A gene to introduce a corn rootworm protease recognition site into the Cry3A protein. In addition, the cry3A coding sequence was optimized to accommodate the preferred codon usage for maize. Except for the introduction of the protease recognition site, the amino acid sequence of the mCry3A protein is identical to that of the native Cry3A protein expressed in Colorado potato beetle resistant potatoes which have been authorized by the CFIA for unconfined release and livestock feed. This protein has been shown to be non-toxic to humans, other vertebrates and invertebrates.

The *mcry3A* gene is expressed in event MIR604 using a promoter which confers preferential expression of the mCry3A protein in the roots of corn. Tissue samples were collected at various growth stages from two event MIR604 hybrids grown at a representative US field trial site. Levels of mCry3A protein were evaluated by enzyme-linked immunosorbent assays. Average mCry3A levels in both hybrids across all stages, expressed in micrograms mCry3A protein per gram dry weight tissue ( $\mu\text{g/g}$ ), ranged from ca. 5-26  $\mu\text{g/g}$  in leaves, 7-25  $\mu\text{g/g}$  in roots and 7-24  $\mu\text{g/g}$  in whole plants. Average levels measured in kernels from both hybrids at senescence were ca. 0.85  $\mu\text{g/g}$ . No mCry3A protein was detected in pollen from the MIR604 hybrids.

The mCry3A protein was shown to degrade readily in the soil. In soil degradation experiments, the average time to degrade 50% of the initial soil concentration of mCry3A protein was 7.6 days.

The mCry3A protein expressed in corn event MIR604 kernels was purified from leaves and characterized. The identity of the purified protein was confirmed by Western immunoblot analysis and insecticidal activity.

The levels of mCry3A protein in corn MIR604 tissues were too low to extract sufficient amounts for evaluation of environmental and feed safety. To obtain sufficient quantities of mCry3A protein for safety studies, it was

necessary to express the *mcry3A* gene in an *E. coli* production system. The equivalency of the plant-produced protein to the *E. coli*-produced protein was evaluated by comparing their molecular weight, immunological reactivity, insecticidal activity and glycosylation status. Based on the results, both proteins were found to be equivalent.

The potential mammalian toxicity and allergenicity of the mCry3A protein was evaluated. The mCry3A protein lacks sequence similarity to known allergens and toxins. No adverse effects were observed when mCry3A protein was ingested by mice at a dose of 2,377 mg/kg body weight. *In vitro* digestive fate studies have shown that the protein is rapidly degraded in simulated gastric fluid, unlike protein allergens which are normally resistant to digestion. The mCry3A protein expressed in event MIR604 is not glycosylated. Incubation for 30 minutes at 95°C or 65°C resulted in complete or partial inactivation of mCry3A protein, respectively.

## 2. Expression of Phosphomannose Isomerase

The *pmi* gene from *Escherichia coli*, which encodes the enzyme phosphomannose isomerase (PMI) was introduced in corn event MIR604. Expression of PMI was used as a selectable marker during the process of developing corn event MIR604. Maize cells producing PMI can utilize mannose as a primary carbon source, whereas cells lacking the *pmi* gene fail to proliferate in a mannose-based culture medium. Expression of the PMI protein is of no agronomic or industrial interest and was only used during the process of regenerating plant material following transformation. Insert sequencing showed that two base-pair changes occurred in the PMI encoding sequence, resulting in two amino-acid changes in the protein sequence. These two amino-acid substitutions have no apparent effect on the functionality of the PMI protein.

The *pmi* gene expressed in corn event MIR604 is linked to a constitutive promoter. Tissue samples were collected at various growth stages from two MIR604 hybrids grown at a representative US field trial site. PMI protein was detected at low levels in most plant tissues analyzed. The highest levels were detected in silk tissue at anthesis (< 0.3-6.8 µg/g) and pollen (3.9 - 5.2 µg/g).

Due to low expression of PMI protein in corn event MIR604, it was not feasible to extract sufficient amounts of PMI protein from plant tissues for safety studies. It was necessary to express the *pmi* gene in an *E. coli* production system to obtain sufficient quantities of PMI protein. The PMI protein produced in *E. coli* contains 16 additional amino-acids at the N-terminus of the protein (derived from the expression vector) and differs by two amino-acids from the PMI protein expressed in MIR604 corn. The equivalency of the MIR604 corn-produced PMI protein to the *E. coli*-produced PMI was evaluated by comparing their immunoreactivity, glycosylation status and enzymatic activity. Based on the results, both proteins were found to be nearly equivalent, with no significant differences.

The potential toxicity and allergenicity of the PMI protein was evaluated. It is likely that small amounts of PMI proteins from various sources have always been present in the food and feed supply due to the ubiquitous occurrence of PMI proteins in nature, including bacteria, yeast, food plants and animals. PMI proteins are present in many mammalian tissues and in humans. Syngenta Seeds Inc. investigated amino acid sequence homology of PMI protein produced in corn event MIR604 with known protein allergens. There is one region of sequence homology of 8 contiguous identical amino acids between the PMI protein and a known allergen, alpha-parvalbumin from *Rana species*. Further investigation using sensitive serum screening technology demonstrated that this sequence identity is not biologically relevant and has no implication for the potential allergenicity of the PMI protein. The PMI protein does not possess significant amino acid sequence similarities to other allergens. *In vitro* digestive fate studies have shown that the PMI protein is rapidly degraded in simulated gastric and intestinal fluids unlike protein allergens which are normally resistant to digestion. The PMI protein is inactivated following incubation at 65°C for 30 minutes and the PMI protein produced in corn event MIR604 is not glycosylated, unlike many known allergens, providing additional evidence that PMI protein does not have the properties of known allergens.

Syngenta Seeds Inc. investigated amino acid sequence homology of PMI protein produced in corn event MIR604 with known protein toxins and found that the PMI protein does not possess significant amino acid sequence similarities to known toxins. Furthermore, no adverse effects were observed when the *E. coli*-produced PMI protein was ingested by mice at a dose of 3,080 mg/kg body weight.

Based on the above, the PMI protein is unlikely to be a toxin or an allergen.

## 3. Development Method

The *mcry3A* and *pmi* genes were introduced in corn event MIR604 via *Agrobacterium*-mediated transformation of immature maize embryos derived from a proprietary maize line. Embryos producing embryogenic callus were transferred to cell culture medium containing mannose. Event MIR604 was identified as a successful transformant and was chosen for further development.

## 4. Stable Integration into the Plant Genome

Molecular characterization by Southern blot analysis demonstrated that corn event MIR604 contains one intact copy of the *mcry3A* and *pmi* gene cassettes inserted at a single site in the corn genome. No additional elements, including intact or partial DNA fragments of the *mcry3A* or *pmi* cassette or backbone sequences from the plasmid vector, linked or unlinked to the intact insert, were detected in corn event MIR604. Sequencing of the introduced DNA confirmed the organization of the genetic elements and revealed that some truncation occurred at the right border and left border of the insert. These deletions have no effect on the functionality of the DNA insert and this phenomenon has been previously observed with *Agrobacterium* transformation. In addition, three single nucleotide changes were also identified in the insert. One of the changes occurred within the promoter driving the expression of the *mcry3A* gene. The remaining two changes occurred with the PMI coding sequence, resulting in two amino acid substitutions. However, these nucleotide changes had no effect on the functionality of the insert.

The inheritance pattern of the *mcry3A* and *pmi* genes within a segregating generation of event MIR604 showed that both genes segregate according to Mendelian rules of inheritance for a single genetic locus. Concentrations of mCry3A and PMI proteins in event MIR604 tissues were measured across four generations and the results indicate stability of mCry3A and PMI protein expression across generations.

#### IV. Criteria for the Environmental Assessment

##### 1. Potential of Corn event MIR604 to Become a Weed of Agriculture or be Invasive of Natural Habitats

The biology of corn, described in the CFIA Biology Document BIO1994-11, shows that unmodified plants of this species are not invasive of unmanaged habitats in Canada. Corn does not possess the potential to become weedy due to the lack of seed dormancy, the non-shattering nature of corn cobs and the poor competitive ability of seedlings. According to the information provided by Syngenta Seeds Inc., corn event MIR604 was determined to be similar to unmodified corn in this respect.

MIR604 corn hybrids were tested at 13 locations in 2002 and 15 locations in 2003 in the United States corn belt. A total of 16 agronomic traits were evaluated. These agronomic traits covered a broad range of characteristics that encompass the entire life cycle of the maize plant and included data assessing seedling emergence, plant height, time to reproduction, lodging, susceptibility to various corn pests and pathogens, and yield characteristics. For the majority of agronomic traits, no statistically significant differences between MIR604 hybrids and their non-transformed isogenic counterparts were observed. Although instances of statistically significant differences between MIR604 and control hybrids were observed for some traits, there were no consistent trends in the data across locations, MIR604 hybrids or years that would indicate that any of these differences were due to the genetic modification. As MIR604 hybrids experience less root feeding damage than controls, some significant agronomic and morphological differences were to be expected depending upon the trait being assessed and combination of pest pressure and environmental conditions experienced at each trial location.

Additionally, specific disease trials conducted in 2002 and 2003 indicated that MIR604 hybrids had no altered susceptibility to corn pathogens compared to control hybrids. No altered susceptibility to non-rootworm insect pests was recorded over the course of the agronomic trials.

The results showed no biologically meaningful differences between corn MIR604 hybrids and their isogenic non-transgenic counterparts, other than resistance to corn rootworm feeding damage. No competitive advantage was conferred to corn event MIR604 by the expression of the mCry3A and PMI proteins, other than that conferred by resistance to rootworms. The introduction of these novel traits did not make corn MIR604 weedy or invasive of natural habitats since none of the corn reproductive or growth characteristics were modified, and MIR604's tolerance to abiotic and biotic stresses was unchanged except for the introduced trait.

The above considerations led the CFIA to conclude that corn event MIR604 has no increased weediness or invasiveness potential compared to currently commercialized corn varieties.

##### 2. Potential for Gene Flow from Corn event MIR604 to Wild Relatives Whose Hybrid Offspring May Become More Weedy or More Invasive

The biology of corn, as described in CFIA Biology Document BIO1994-11, indicates that there are no wild relatives in Canada that can hybridize with corn. None of the data submitted by Syngenta Seeds Inc. on corn MIR604 indicated any change in sexual compatibility as a result of the genetic modification.

The CFIA has therefore concluded that gene flow from corn event MIR604 to wild relatives is not possible in Canada.

##### 3. Altered Plant Pest Potential of Corn event MIR604

The expression of the mCry3A  $\delta$ -endotoxin and PMI phosphomannose isomerase is unrelated to plant pest potential, and corn is not considered a plant pest in Canada. Field evaluation of MIR604 corn hybrids did not show any increase or decrease in susceptibility to disease or insect stressors, other than to rootworms, compared to unmodified corn counterparts. Rootworm damage is not known to be a principal factor restricting the establishment or distribution of corn in Canada.

The CFIA has, therefore, determined that the corn event MIR604 does not display any altered pest potential compared to currently commercialized corn varieties.

#### 4. Potential Impact of Corn event MIR604 on Non-Target Organisms

Literature reports and studies to support the safety of potatoes expressing Cry3A proteins indicate that Cry3A *Bacillus thuringiensis*  $\delta$ -endotoxins are active only against specific coleopteran insects, and are not toxic to humans, other vertebrates, and non-coleopteran invertebrates. The mCry3A protein has a similar spectrum of activity to the native Cry3A, but with enhanced toxicity to Northern and Western corn rootworms, both major coleopteran pests of corn. There are no coleopteran species currently listed by the [Committee on the Status of Endangered Wildlife in Canada](#) as being a threatened or endangered species. (Please refer to <http://www.cosewic.gc.ca/index.htm> for more information). As mCry3A is not detectable in pollen of MIR604 corn, MIR604 corn poses minimal risk to pollinators and non-target pollen consumers.

Syngenta Seeds Inc. has submitted data from dietary toxicity studies on the effect of the bacterial mCry3A protein on non-target invertebrates, including the honeybee (*Apis mellifera*), insidious flower bug (*Orius insidiosus*), seven spot ladybird (*Coccinella septempunctata*), ground beetle (*Poecilus cupreus*), rove beetle (*Aleochara bilineata*) and earthworm (*Eisenia foetida*). In all cases, the mCry3A protein was demonstrated to be safe to these indicator species at doses exceeding 5 times the estimated environmental concentration of mCry3A in the diet of non-target invertebrates feeding on MIR604 tissues or exposed to MIR604 corn via their preys.

Monarch butterfly (*Danaus plexippus*) larvae feeding on milkweed plants may be exposed to corn pollen drifting from adjacent corn plants. As the activity of mCry3A protein is restricted to some Coleopteran species (*D. plexippus* is a Lepidopteran) and mCry3A is not detectable in pollen of MIR604 corn, MIR604 corn poses no risk to the monarch butterfly.

Data was also submitted on non-target vertebrates including the mouse, the bobwhite quail and the rainbow trout. No harmful effects were detected when mice and quails were exposed to a single oral dose of 2,377 mg mCry3A/kg body weight and 652 mg mCry3A/kg body weight, respectively. These doses represent 2,600 times and 1,400 times the worst-case daily dose of mCry3A to mammals and birds feeding on MIR604 grain, respectively. No harmful effects were detected when juvenile rainbow trout were fed a fish diet containing 50% MIR604 corn grain for 28 days.

Phosphomannose isomerase is a ubiquitous enzyme involved in carbohydrate metabolism. Phosphomannose isomerases of varying degrees of amino acid homology to PMI expressed in corn event MIR604 occur widely in nature. Phosphomannose isomerases have been detected in some crop species, mammals, humans, yeast, fungi and bacteria. Species that will be exposed to PMI from MIR604 corn tissues are highly likely to have prior exposure to similar PMI proteins. No harmful effects of such exposure are known or expected. The PMI protein expressed in corn event MIR604 is not homologous to any known toxins. Additionally, the bacterially-expressed PMI protein showed lack of acute toxicity to the mouse at a single oral dose of 3,080 mg PMI kg body weight.

Field evaluations showed that MIR604 corn is more resistant to corn rootworm than unmodified corn, but not more resistant to other pest insects or pathogens.

Composition analyses showed that the levels of key nutrients and anti-nutrients in corn MIR604 grain and forage are comparable to those in commercial corn varieties.

Based on the above, the CFIA has determined that, compared to current commercial corn varieties, the unconfined release of corn event MIR604 will not result in altered impacts on non-target organisms, including humans.

#### 5. Potential Impact of Corn event MIR604 on Biodiversity

Corn event MIR604 has no novel phenotypic characteristics that would extend its range beyond the current geographic range of corn production in Canada. Since corn has no wild relatives with which it can outcross in Canada, there will be no transfer of the novel traits to other species in unmanaged environments. In addition the novel traits were determined to pose minimal risks to non-target organisms.

At present, the use of chemical insecticides to control rootworm is permitted in Canada. Currently, crop rotation represents the principal method of rootworm control in Canada. MIR604 corn provides an alternative method to existing methods of control of corn rootworm. Therefore, the reduction in local pest species as a result of the release of MIR604 corn does not present a significant change from existing agricultural practices.

The CFIA has therefore concluded that the potential impact on biodiversity of corn event MIR604 is equivalent to that of currently commercialized corn varieties.

## **6. Potential for Development of Rootworm Resistance to Corn event MIR604**

In order to significantly minimize the likelihood of the development of insect pest resistance to PNTs expressing novel insect resistance, the CFIA requires that an insect resistance management (IRM) plan be implemented for these products. Coleopteran insects have a significant ability to develop resistance to conventional chemical insecticides. Therefore it is reasonable to expect that resistance to the insecticidal properties of corn event MIR604 may develop. The following IRM design is intended to reduce or delay corn root worm (CRW) resistance to the mCry3A protein. A component of the IRM strategy that will be used with MIR604 corn is the establishment of a refuge of CRW-susceptible corn within or adjacent to the MIR604 corn field. Should resistant insects occur, they would then be able to mate with susceptible insects to keep the frequency of resistance genes diluted in the insect population.

CFIA believes that sound management practices and IRM strategies can significantly reduce and delay the development of mCry3A protein resistant CRW populations. However the CRW populations must be monitored for the development of resistance in a regular and consistent manner.

CFIA understands that Syngenta Seeds Canada Inc. has developed, and will implement, an IRM plan that includes the following key components:

1. The use of structured refugia to provide a population of corn rootworms that have not been exposed to the mCry3A protein and are available to reproduce with potentially resistant rootworms which may emerge from the MIR604 crop.
2. The early detection of rootworm populations resistant to the corn-expressed insecticidal protein is extremely important. Close monitoring for the presence of such populations, in rootworm-resistant corn fields and surrounding areas, is therefore warranted. Monitoring includes the development of appropriate detection tools such as visual field observations and laboratory bioassays, education of growers, reporting schedules, and mitigation procedures in case of resistance development.
3. Education tools will be developed and provided to all growers, district managers and field managers. These will include information on product performance, resistance management, monitoring procedures and timetables, detection protocols for resistant rootworm individuals, instructions to contact Syngenta Seeds Inc. and strategies to be followed if unexpected levels of rootworm damage occur.
4. Syngenta Seeds Canada Inc. will have documented procedures in place for responding to these reported instances of unexpected rootworm damage. These procedures will include, where warranted, the collection of plant tissue and rootworms and use of appropriate bioassays to evaluate suspected mCry3A resistant individuals, and a protocol for immediate action to control resistant individuals.
5. Detection of confirmed resistant rootworm populations and mitigation measures will be immediately reported to CFIA.
6. Integrated Pest Management practices will be promoted, such as prediction of infestation problems from field histories.

Research related to the proposed IRM plan is ongoing, and as research progresses, the new information will be used to determine if the present IRM plan should be maintained in its present form, or if it will be modified. Therefore, the renewal of the present three year authorization will be contingent upon Syngenta Seeds Canada Inc. demonstrating significant progress in research related to rootworm resistance management.

Note: The Plant Biosafety Office periodically audits compliance with the IRM requirements.

## **V. Criteria for the Livestock Feed Assessment**

### **1. Potential Impact of Corn event MIR604 on Livestock Nutrition**

## Nutritional Composition and Anti-Nutritional Factors

### Nutritional Composition:

Nutritional data was obtained from four corn hybrid pairs containing event MIR604 hybrids (D, F, E3 and E4) and their respective parental controls (C, E, E1, and E2). Forage and grain samples were collected from replicated plots at 12 locations over the 2002 and 2003 growing seasons (two hybrid pairs each). Samples in each year were analyzed for proximate, amino acids, fatty acids, minerals, vitamins, phytosterols and secondary metabolites (ferulic acid, p-coumaric acid and furfural). No statistically significant differences were observed between MIR604 forage and its controls for proximate, Acid Detergent Fibre (ADF), Neutral Detergent Fibre (NDF), Ca and P, except for ash in 2002 (one hybrid pair) and protein in 2003 (one hybrid pair). All means were within ranges of literature values. No statistically significant differences were observed between MIR604 grain and its controls for proximate and fibre in 2002. Fat (one hybrid pair), protein, moisture, and ADF (one hybrid pair), were statistically significantly higher in MIR604 grain than controls in 2003, but these analytes were within literature ranges. Statistically significant differences were observed in MIR604 hybrids and controls for oleic (one hybrid pair) and stearic (both hybrid pairs) in 2002. In 2003, statistically significant differences were observed between MIR604 grain and controls for stearic, oleic, linoleic and linolenic (one hybrid pair), however fatty acids were within ranges of literature values. No statistically significant differences were observed for amino acids analyzed in 2002, except for tyrosine which was lower in MIR604 (F) than control (E), and methionine which was higher in MIR604(D) than control (C). Statistically significant differences were observed between MIR604 grain and their controls for aspartic acid, threonine, serine, glutamic acid, alanine, tyrosine (one hybrid pair), valine, isoleucine, leucine, cystine (one hybrid pair) and phenylalanine in 2003, however all means were within literature values. Statistically significant differences were observed between MIR604 and controls for Zn (both hybrid pairs) in 2002 and for Cu, Fe, Mg, Mn, P (one hybrid pair) Ca, (both hybrids pairs) in 2003. Statistically significant differences were observed for Vitamins B1, B2, E, niacin (one hybrid pair), pyridoxine,  $\beta$ -carotene, cryptoxanthin and  $\gamma$ -tocopherol between MIR604 grain and controls. All minerals and vitamins analyzed were within literature ranges. Campesterol and stigmasterol were significantly higher in MIR604 hybrids than controls, while there were no statistically significant differences in  $\beta$ -sitosterol between MIR604 and controls for  $\beta$ -sitosterol. Ferulic acid and p-coumaric acid were statistically significantly lower in MIR604 compared to their controls, however the ranges were within literature values.

### Anti-nutritional factors:

Phytic acid, raffinose and trypsin inhibitor were analyzed in MIR604 corn (four hybrids) and compared to parental controls. The trypsin inhibitor was not significantly different between MIR604 and their controls. Phytic acid and raffinose had some values below the limits of quantification for the assay and therefore were not subjected to statistical analyses.

### Nutrient Bioavailability:

Syngenta conducted a 49-day broiler chicken study to evaluate whether transgenic MIR604(+ve) corn grain had any adverse dietary effects on broiler chickens when compared with those fed non-transgenic corn (MIR604(-ve)). 900 birds were distributed into 36 pens (25 birds/pen), assigned in a randomized complete block design. No statistically significant differences were observed in % survival of birds on MIR604(+ve and MIR604(-ve) diets. Body weights and feed conversion efficiencies of birds were similar between test and control diets. Carcass analyses showed no statistically significant differences between test and control except for thigh meat weight among female birds. In conclusion, the trial supports the lack of deleterious effects on animal performance and health associated with the diets from MIR604 grain when compared to the non-transgenic corn.

The evidence provided by Syngenta supports the conclusion that the nutritional composition of MIR604 corn is substantially equivalent to conventional corn varieties.

## 2. Potential Impact of Corn event MIR604 on Livestock and Workers/Bystanders

Corn is not known for the production of endogenous allergens and the transformation event which produced Event MIR604 would not be expected to induce their synthesis.

The mode of action and its structural similarity to *Bacillus thuringiensis* proteins, including Cry3A, for which human and animal safety have been previously established support the prediction that no adverse health effects will result from exposure to the modified Cry3A (mCry3A) protein encoded by the *mcry3A* synthetic gene derived from *Bacillus thuringiensis* subsp. *tenebrionis*. Acute oral toxicity studies demonstrated non-observed-effect-level (NOEL) values of 2377 mg/kg-bw/day for mCry3A and 3080 mg/kg-bw/day for the bacterial PMI protein which are much higher values than the predicted worst case exposure of 1.76 mg/kg-bw/day for mCry3A and 0.086 mg/kg-bw/day for PMI in starter weaner pigs. The proteins mCry3A and PMI have been demonstrated to be heat labile and rapidly digested under conditions present in the gastrointestinal tract. Toxicity findings indicate that MIR604 which produces mCry3A and PMI proteins is not expected to result in adverse toxicological effects in livestock fed MIR604 corn. These proteins have shown no sequence similarities with known allergens or toxins which would indicate potential allergenicity or toxicity issues.

The history of use and literature suggest that the bacterial *Bacillus thuringiensis* Cry proteins are not toxic to humans and other vertebrates. The low mammalian toxicity of *Bacillus thuringiensis* microbial insecticide mixtures containing Cry proteins has been demonstrated over the last 40 years.

Based on the predicted exposure levels and the results of the above tests, no significant risk to livestock and

workers/bystanders is expected from exposure to the modified Cry3A (mCry3A) protein.

## VI. New Information Requirements

If at any time, Syngenta Seeds Canada Inc. becomes aware of any information regarding risk to the environment, including risk to human or animal health, which could result from release of corn event MIR604 materials in Canada or elsewhere, Syngenta Seeds Canada Inc. will immediately provide such information to the CFIA. On the basis of such new information, the CFIA will re-evaluate the potential impact of event MIR604 on the environment, livestock and human health, and may re-evaluate its decision with respect to the livestock feed use and environmental release authorizations of corn event MIR604.

## VII. Regulatory Decision

Based on the review of the data and information submitted by Syngenta Seeds, Inc., and through comparisons of corn event MIR604 with unmodified corn counterparts, the Biotechnology Environmental Release Assessment Unit, CFIA, has concluded that the novel genes and their corresponding traits do not confer to corn event MIR604 any characteristic that would result in unintended environmental effects following unconfined release.

Based on the review of submitted data and information by Syngenta Seeds Inc., including comparisons of corn event MIR604 with its unmodified corn counterparts, the Feed Section of the Animal Health and Production Division has concluded that the introduced genes and their corresponding traits will not confer to corn event MIR604 any characteristic that would raise any concerns regarding the safety or nutritional composition of corn event MIR604. Grain corn, its byproducts and corn oil are currently listed in Schedule IV of the *Feeds Regulations* and are, therefore, approved for use in livestock feeds in Canada. Corn event MIR604 has been assessed and found to be as safe as and as nutritious as traditional corn varieties. Corn event MIR604 and its products are considered to meet present ingredient definitions and are approved for use as livestock feed ingredients in Canada.

**Unconfined release into the environment and use as livestock feed of corn event MIR604 is therefore authorized by Plant Biosafety Office of the Plant Products Directorate and the Feed Section of the Animal Health and Production Division as of July 20, 2007. The authorization is limited to three years. Renewal of the three year authorization is conditional upon the submission of additional research results related to rootworm resistance management. Any corn lines derived from event MIR604 may also be released into the environment and used as livestock feed, provided no inter-specific crosses are performed, provided the intended uses are similar, provided it is known based on characterization that these plants do not display any additional novel traits and are substantially equivalent to currently grown corn varieties in Canada in terms of their potential environmental impact and livestock feed safety, provided the novel genes are expressed at a level similar to that of the authorized line and provided the insect resistance management requirements described in the present document are applied.**

Corn event MIR604 is subject to the same phytosanitary import requirements as its unmodified counterpart.

Please refer to Health Canada's Decisions on Novel Foods for a description of the food safety assessment of corn event MIR604. The food safety decisions are available at the following [Health Canada web site](http://www.hc-sc.gc.ca/fn-an/gmf-agm/appro/index_e.html):  
[http://www.hc-sc.gc.ca/fn-an/gmf-agm/appro/index\\_e.html](http://www.hc-sc.gc.ca/fn-an/gmf-agm/appro/index_e.html)

This bulletin is published by the Canadian Food Inspection Agency. For further information, please contact the Plant Biosafety Office or the Feed Section at:

Plant Biosafety Office  
Plant Products Directorate  
59 Camelot Drive  
Ottawa, Ontario K1A 0Y9  
Telephone: 613-225-2342

Feed Section  
Animal Health and Production Division  
Animal Products Directorate  
59 Camelot Drive  
Ottawa, Ontario K1A 0Y9  
Telephone: 613-225-2342

  
[Top of Page](#)

[Important Notices](#)