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Canadian Food Inspection Agency

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Decision Document DD2016-118 Determination of the Safety of Syngenta Canada Inc.'s Corn (Zea mays L.) Event MZIR098

This Decision Document has been prepared to explain the regulatory decisions reached under Directive 94-08 – Assessment Criteria for Determining Environmental Safety of Plants with Novel Traits, its companion document BIO1994-11 – The Biology of Zea mays (L.) (Corn) and Section 2.6 – Guidelines for the Assessment of Novel Feeds: Plant Sources, of Chapter 2 of the RG-1 Regulatory Guidance: Feed Registration Procedures and Labelling Standards.

The Canadian Food Inspection Agency (CFIA) – specifically the Plant Biosafety Office of the Plant Health and Biosecurity Directorate, the Plant and Biotechnology Risk Assessment Unit of the Plant Health Science Directorate and the Animal Feed Division of the Animal Health Directorate – has evaluated information submitted by Syngenta Canada Inc. This information concerns the insect resistant and herbicide-tolerant corn event MZIR098. 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 or nutrition concerns when compared to corn varieties currently grown and permitted to be used as livestock feed in Canada.

Taking into account these evaluations, unconfined release into the environment and use as livestock feed of corn event MZIR098 is therefore authorized by the Plant Biosafety Office of the Plant Health and Biosecurity Directorate and the Animal Feed Division of the Animal Health Directorate, respectively, as of August 9th, 2016. Any corn lines derived from the event MZIR098 may also be released into the environment and used as livestock feed, provided that:

(i) no inter-specific crosses are performed,
(ii) the intended use (s) are similar,
(iii) it is known based on characterization that these plants do not display any additional novel traits and are substantially equivalent to corn varieties that are currently grown and permitted to be used as livestock feed in Canada, in terms of their potential environmental impact and livestock feed safety and nutrition, and
(iv) the novel genes are expressed at levels similar to that of the authorized line.

With respect to its unconfined release into the environment, cultivation of corn event MZIR098 is subject to insect resistance management requirements.

Additionally, with respect to its unconfined release into the environment, an appropriate herbicide tolerance management plan should be implemented.

Before corn event MZIR098 is cultivated in Canada as an individual event or in combination with other corn events in stacked/pyramided products, Syngenta Canada Inc. must submit a herbicide tolerance management plan to the CFIA.

Corn event MZIR098 is subject to the same phytosanitary import requirements as unmodified corn varieties. Corn event MZIR098 must also meet the requirements of other Canadian legislation, including but not limited to the requirements set out in the Food and Drugs Act and the Pest Control Products Act.

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

(publié aussi en français)

August 9, 2016

This bulletin is published by the Canadian Food Inspection Agency. For further information, please contact the

Plant Biosafety Office or the Animal Feed Division by visiting the contact page.

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I. Brief Identification of the Modified Plant

Designation of the Modified Plant:	Corn event MZIR098, OECD Unique Identifier SYN-ØØØ98-3
Applicant:	Syngenta Canada Inc.

Plant Species:	Corn (Zea mays L.)
Novel Traits:	Resistance to the coleopteran pests Western corn rootworm (Diabrotica virgifera virgifera) and Northern corn rootworm (Diabrotica longicornis barberi); tolerance to glufosinate-ammonium herbicide
Trait Introduction Method:	Agrobacterium-mediated transformation
Intended Use of the Modified Plant:	Corn event MZIR098 is intended for human consumption and livestock feed uses. Corn event MZIR098 is not intended to be grown outside the normal production area for corn in Canada.

II. Background Information

Syngenta Canada Inc. has developed a corn event that is resistant to Western corn rootworm (Diabrotica virgifera virgifera) and Northern corn rootworm (Diabrotica longicornis barberi), and is tolerant to glufosinate-ammonium. Corn event MZIR098 was developed using recombinant deoxyribonucleic acid (rDNA) technology to introduce the ecry3.1Ab, mcry3A and phosphinothricin N-acetyltransferase (pat) genes. The ecry3.1Ab gene is derived from Bacillus thuringiensis subspecies tenebronis and B. thuringiensis subspecies kurstaki, and encodes the eCry3.1Ab protein that protects corn against Western and Northern corn rootworms. The mcry3A gene is derived from B. thuringiensis subspecies tenebronis, and encodes the mCry3A protein that protects corn against Western and Northern corn rootworms. The eCry3.1Ab and mCry3A proteins are also present in corn event 5307 and corn event MIR604, respectively. Both corn events were previously authorized for unconfined environmental release and animal feed use in Canada (DD2013-96 and DD2007-68). The pat gene is derived from the soil bacterium Streptomyces viridochromogenes,and encodes the phosphinothricin N-acetyltransferase (PAT) protein, which confers tolerance to the herbicide glufosinate-ammonium. The PAT protein is present in corn event Bt11 and corn event MZHG0JG. Both corn events were previously authorized for unconfined environmental release and animal feed use in Canada (DD1996-12 and DD2016-116).

Syngenta Canada Inc. has provided information on the identity of corn event MZIR098; a detailed description of the transformation method; and information on insert copy number and intactness, levels of protein expression in the plant and the role of the inserted sequences. The novel proteins were identified and characterized. Information 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 livestock.

A hybrid of corn event MZIR098 (hereafter referred to as the corn event MZIR098 hybrid) and an unmodified corn variety (hereafter referred to as the the unmodified control corn hybrid)were field tested in the United States (US) at 8 sites in 2013. The locations of these trials share similar environmental and agronomic conditions to corn production areas in Canada and were considered representative of major Canadian corn growing regions. The corn event MZIR098 hybrid was compared to an unmodified control corn hybrid with a similar genetic background. Several reference corn hybrids were also included in the field trials to establish ranges of comparative values that are typical of currently grown corn varieties in Canada.

Agronomic characteristics of the corn event MZIR098 hybrid, including early stand count (pre-thinning and post-thinning), days to 50% pollen shed, days to 50% silking, stay green rating, ear height, plant height, dropped ears, stalk lodged plants, root lodged plants, final stand count, grain moisture, test weight and grain yield, were compared to those of the unmodified control corn hybrid and to the ranges established by the reference corn hybrids.

Nutritional components of grain and forage from the corn event MZIR098 hybrid, such as protein, fat, moisture, ash, carbohydrates, fiber, amino acids, fatty acids, vitamins, minerals, secondary metabolites and anti-nutrients, were compared with those of the unmodified control corn hybrid and to the range established by the reference corn hybrids.

The Plant and Biotechnology Risk Assessment (PBRA) Unit of the Plant Health Science Directorate, CFIA, has reviewed the above information, in light of the assessment criteria for determining environmental safety of PNTs, as described Directive 94-08 – Assessment Criteria for Determining Environmental Safety of Plants with Novel Traits. The PBRA Unit has considered:

- the potential for corn event MZIR098 to become a weed of agriculture or to be invasive of natural habitats;
- the potential for gene flow from corn event MZIR098 to sexually compatible plants whose hybrid offspring may become more weedy or more invasive;
- the potential for corn event MZIR098 to become a plant pest;
- the potential impact of corn event MZIR098 and its gene products on non-target organisms, including humans; and
- the potential impact of corn event MZIR098 on biodiversity.

The Animal Feed Division (AFD) of the CFIA has also reviewed the above information with respect to the assessment criteria for determining the safety and nutrition of livestock feed, as described in Section 2.6 – Guidelines for the Assessment of Novel Feeds: Plant Sources, of Chapter 2 of the RG-1 Regulatory Guidance: Feed Registration Procedures and Labelling Standards.

The AFD has considered both intended and unintended effects and similarities and differences between corn event MZIR098 and unmodified corn hybrids relative to the safety and nutrition of feed ingredients derived from corn event MZIR098 for their intended purpose, including:

- the potential impact of corn event MZIR098 on animal health and human safety, as it relates to the potential transfer of residues into foods of animal origin and worker/bystander exposure to the feed; and
- the potential impact of corn event MZIR098 on livestock nutrition.

The AFD has also considered whether feeds derived from corn event MZIR098 meet the definitions and requirements of feeds as listed in Schedule IV of the Feeds Regulations.

III. Description of the Novel Traits

1. Development Method

Corn event MZIR098 was developed through Agrobacterium-mediated transformation of immature Z. mays embryos, and contains the ecry3.1Ab, mcry3A and pat genes and their associated regulatory elements. Transformed cells were selected on the basis of tolerance to glufosinate-ammonium herbicide and regenerated to produce plants. Presence of ecry3.1Ab, mcry3A and pat genes was further confirmed by molecular analysis. Corn event MZIR098 was identified as a successful transformant and was chosen for further development.

2. Resistance to Corn Rootworms (Diabrotica spp.)

B. thuringiensis is a common gram-positive soil-borne bacterium. In its spore forming stage, it produces several protein crystals called Cry proteins. These Cry proteins are insecticidal to susceptible insect species after cleavage by proteases in the insect gut, forming a protease-resistant active fragment, which is the bio-active form of the

proteins. Insecticidal activity is believed to depend on the binding of the active fragment to specific midgut epithelial cell receptors of susceptible insects. Binding of Cry proteins induce pore formation which disrupts osmotic balance, resulting in cell lysis and insect death.

eCry3A.1Ab

The eCry3.1Ab protein is a chimera of the Cry3A protein of B. thuringiensis subspecies tenebrionis, which possesses activity against corn rootworms, and the Cry1Ab protein of B. thuringiensis subspecies kurstaki, which possesses activity against certain lepidopteran insects. The chimeric protein is only active against corn rootworms but not against lepidopteran insects since the portion of the Cry1Ab protein used to create eCry3.1Ab is not from the region responsible for its activity on lepidopteran insects. The eCry3.1Ab protein produced in corn event MZIR098 is the same as the eCry3.1Ab protein produced in corn event 5307 which has already been authorized for unconfined environmental release and livestock feed use in Canada (DD2013-96).

The expression of the eCry3.1Ab protein in corn event MZIR098 is driven by a constitutive promoter. Samples of corn tissues were collected from plants from 4 field trials in the US. The average eCry3.1Ab protein expression in micrograms protein per gram dry weight tissue (µg/g dwt), as evaluated by enzyme-linked immunosorbent assays (ELISA), was as follows: 2 to 334 µg/g dwt in leaves, 2 to 96 µg/g dwt in roots, 3 to 221 µg/g dwt in whole plant, 1 to 6 µg/g dwt in kernels and less than the limit of detection (LOD) in pollen.

The potential allergenicity and mammalian toxicity of the eCry3.1Ab protein were evaluated. The weight of evidence indicates that the eCry3.1Ab protein is unlikely to be allergenic, based on the following information. The source of the ecry3.1Ab gene, B. thuringiensis, is not commonly associated with allergenicity, and the eCry3.1Ab protein amino acid sequence lacks relevant similarities to known allergens. The eCry3.1Ab protein produced in an E. coli production system, previously evaluated in support of safety of corn event 5307, was shown experimentally to be rapidly degraded in simulated gastric fluid and to be denatured by heat, and this protein expressed in corn event MZIR098 was shown experimentally to be non-glycosylated. It was also concluded that the eCry3.1Ab protein is unlikely to be toxic to mammals because it lacks a mode of action to suggest that it is intrinsically toxic to mammals and because the eCry3.1Ab protein amino acid sequence lacks relevant similarities to known mammalian toxins. Additionally, data submitted in support of corn event 5307 safety demonstrated no adverse effects when the E. coli-produced eCry3.1Ab protein was ingested by mice at a dose of approximately 2000 mg/kg body weight. For a more detailed discussion of the potential allergenicity and toxicity of the eCry3.1Ab protein, see Section V, part 2: Potential Impact of Corn Event MZIR098 on Animal Health and Human Safety as it Relates to the Potential Transfer of Residues into Foods of Animal Origin and Worker/Bystander Exposure to the Feed.

mCry3A

The mcry3A gene is derived from the cry3A gene originally isolated from B. thuringiensis subspecies tenebrionis and possesses activity against Western and Northern corn rootworms. The mCry3A protein produced in corn event MZIR098 is the same as the mCry3A protein produced in corn event MIR604 which was previously authorized for unconfined environmental release and livestock feed use in Canada (DD2007-68).

The expression of the mCry3A protein in corn event MZIR098 is driven by a constitutive promoter. Samples of corn tissues were collected from plants from 4 field trials in the US. The average mCry3A protein expression in µg/g dwt, as evaluated by ELISA, was as follows: 4 to 114 µg/g dwt in leaves, 5 to 111 µg/g dwt in roots, 9 to 95 µg/g dwt in whole plant, 7 to 23 µg/g dwt in kernels and 294 to 309 µg/g dwt in pollen.

The potential allergenicity and mammalian toxicity of the mCry3A protein to livestock and non-target organisms were evaluated. The weight of evidence indicates that the mCry3A protein is unlikely to be allergenic, based on the following information. The source of the mcry3A gene, B. thuringiensis, is not commonly associated with allergenicity, and the mCry3A protein amino acid sequence lacks relevant similarities to known allergens. The mCry3A protein produced inan E. coli production system, previously evaluated in support of safety of corn event

MIR604, was shown experimentally to be rapidly degraded in simulated gastric fluid and to be denatured by heat, and this protein expressed in corn event MZIR098 was shown experimentally to be non-glycosylated. It was also concluded that the mCry3A protein is unlikely to be toxic to mammals because it lacks a mode of action to suggest that it is intrinsically toxic to mammals and because the mCry3A protein amino acid sequence lacks relevant similarities to known mammalian toxins. Additionally, data submitted in support of corn event MIR604 safety demonstrated no adverse effects when the E. coli-produced mCry3A protein was ingested by mice at a dose of approximately 2,377 mg/kg body weight. For a more detailed discussion of the potential allergenicity and toxicity of the mCry3A protein, see Section V, part 2: Potential Impact of Corn Event MZIR098 on Animal Health and Human Safety as it Relates to the Potential Transfer of Residues into Foods of Animal Origin and Worker/Bystander Exposure to the Feed.

3. Tolerance to Glufosinate-Ammonium Herbicide

Glufosinate-ammonium herbicide inhibits the plant enzyme glutamine synthetase. Inhibiting glutamine synthetase results in reduced glutamine synthesis, and accumulation of lethal levels of ammonia in susceptible plants. Ammonia is produced by plants as a result of normal metabolic processes, but elevated levels of ammonia can interfere with essential plant processes, like photosynthesis, leading to plant death.

Corn event MZIR098 was developed to be tolerant to glufosinate-ammonium herbicide by incorporation of the pat gene. The pat gene encodes the enzyme PAT, which acetylates the primary amino group of glufosinate-ammonium, rendering the herbicide inactive. Introduction of the pat gene into corn event MZIR098 confers commercial-level tolerance to glufosinate-ammonium herbicide.

The pat gene was derived from S. viridochromogenes, a gram-positive soil bacterium. The PAT protein produced in corn event MZIR098 is identical to the native enzyme in terms of its amino acid sequence. Furthermore, the PAT protein expressed in corn event MZIR098 is identical to the PAT protein in events Bt11 (DD1996-12) and MZHG0JG (DD2016-116), which have been authorized for unconfined environmental release and livestock feed use in Canada.

Expression of the PAT protein in corn event MZIR098 is driven by a constitutive promoter. Samples of corn tissues were collected from plants from 4 field trials in the US. The average PAT protein expression in µg/g dwt, as evaluated by ELISA, was as follows: <LOD to 13 µg/g dwt in leaf, <LOD to 3 µg/g dwt in root, <LOD to 7 µg/g dwt in whole plants, <LOD in pollen and kernel.

The potential allergenicity and toxicity of the PAT protein expressed in corn event MZIR098 were evaluated. The weight of evidence indicates that the PAT protein is unlikely to be allergenic, based on the following information. The source of the pat gene, S. viridochromogenes, is not commonly associated with allergenicity and the PAT protein amino acid sequence lacks relevant similarities to known allergens. PAT protein produced inan E. coli production system, previously evaluated in support of safety of corn event MZHG0JG, was shown experimentally to be rapidly degraded in simulated gastric and intestinal fluid and to be denatured by heat, and this protein expressed in corn event MZIR098 was shown experimentally to be non-glycosylated. It was also concluded that the PAT protein is unlikely to be toxic to non-target organisms because it lacks a mode of action to suggest that it is intrinsically toxic to non-target organisms and the PAT protein amino-acid sequence lacks relevant similarities to known toxins. For a more detailed discussion of the potential allergenicity and toxicity of the PAT protein, see Section V, part 2: Potential Impact of Corn Event MZIR098 on Animal Health and Human Safety as it Relates to the Potential Transfer of Residues into Foods of Animal Origin and Worker/Bystander Exposure to the Feed.

4. Stable Integration into the Plant Genome

Molecular characterization by DNA sequencing and Southern blot analysis demonstrated that corn event MZIR098 contains one intact copy of the ecry3.1Ab, mcry3A and pat genes, and their associated regulatory elements inserted at a single site in the corn genome. No additional elements, including intact or partial DNA fragments or backbone sequences from the plasmid vector, linked or unlinked to the intact insert, were detected in corn event MZIR098.

The stability of the insert was verified by Southern blot analysis across five generations in the breeding history of corn event MZIR098. The inheritance pattern of the insert was evaluated by Real-Time PCR across three segregating generations of corn event MZIR098. The combined analysis showed that the insert is stably inherited and segregates according to the Mendelian rules of inheritance for a single genetic locus.

IV. Criteria for the Environmental Assessment

1. Potential for Corn Event MZIR098 to Become a Weed of Agriculture or be Invasive of Natural Habitats

The CFIA biology document BIO1994-11 – The Biology of Zea mays (L.) (Corn) states 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 Canada Inc., corn event MZIR098 was determined not to be significantly different from unmodified corn in this respect.

The CFIA evaluated data submitted by Syngenta Canada Inc. on the reproductive biology and life history traits of corn event MZIR098. As previously mentioned, the corn event MZIR098 hybrid was field tested in the US at 8 sites in 2013. It was determined that the US locations share similar environmental and agronomic conditions to corn production areas in Southern Ontario and Quebec. Therefore, they were considered representative of the major Canadian corn growing regions. During the field trials, the corn event MZIR098 hybrid was compared to an unmodified control corn hybrid with a similar genetic background. Reference corn hybrids were also included in these trials to establish ranges of comparative values that are representative of currently grown corn varieties in Canada. Phenotypic and agronomic traits were evaluated, covering a broad range of characteristics that encompass the entire life cycle of the corn plant. The traits included early stand count (pre-thinning and post-thinning), days to 50% pollen shed, days to 50% silking, stay green rating, ear height, plant height, dropped ears, stalk lodged plants, root lodged plants, final stand count, grain moisture, test weight and grain yield.

No significant differences were observed between the corn event MZIR098 hybrid and the unmodified control corn hybrid in any of the characteristics measured. Some characteristics were not suitable for statistical analysis (early stand count (post-thinning), final stand count, early growth rating, days to 50% pollen shed, days to 50% silking and dropped ears) but were numerically similar when the corn event MZIR098 hybrid and the unmodified control corn hybrid were compared, and within the ranges established from the reference corn hybrids. Therefore the results support a conclusion of phenotypic and agronomic equivalence of corn event MZIR098 to currently grown corn varieties.

Syngenta Canada Inc. provided information on the dormancy and germination of corn event MZIR098 seed under 6 different temperature regimes (10ºC; 25ºC; 30ºC; alternating 10°C for 16 hours followed by 20°C for 8 hours; alternating 10°C for 16 hours followed by 30°C for 8 hours; and alternating 20°C for 16 hours followed by 30°C for 8 hours). Seed germination characteristics were evaluated, including percent germinated seed (normal or abnormal), percent hard seed, percent dead seed and percent dormant seed. The corn event MZIR098 hybrid was compared to the unmodified control corn hybrid, and 3 reference corn hybrids were included to provide a range of comparative values for each germination characteristic. No statistically significant differences were observed between the corn event MZIR098 hybrid and the unmodified control corn hybrid at any temperature regime for percent germinated

seed (normal). There were no statistically significant differences in percent germinated seed (abnormal) (evaluated only under the 25ºC and alternating 20°C for 16 hours followed by 30°C for 8 hours temperature regimes), percent dead seed, or percent dormant seed between the corn event MZIR098 hybrid and the unmodified control corn hybrid. Moreover, a lack of hard seed indicated that corn event MZIR098 does not possess seed dormancy. Therefore, the introduction of the novel traits did not impact the germination of the corn seed and did not confer dormancy to the corn seed.

The response of corn event MZIR098 to abiotic stressors was observed at 8 sites in 2013. The stressors observed included drought, wet soils, nutrient deficiency and wind. No consistent trend in increased or decreased susceptibility to these abiotic stressors was observed in the corn event MZIR098hybridcompared to the unmodified control corn hybrid.

The susceptibility of corn event MZIR098 to corn pests and pathogens was evaluated at 8 sites in 2013. The stressors observed included corn earworm, Fusarium spp., corn rust, corn borer, grasshopper, corn rootworm, gray leaf spot, northern corn blight, Stewart's disease and common smut. No consistent trend in increased or decreased susceptibility to pests or pathogens was observed in the corn event MZIR098 hybridcompared to the unmodified control corn hybrid.

No competitive advantage was conferred to plants of corn event MZIR098, other than that conferred by resistance to corn rootworms and tolerance to glufosinate-ammonium herbicide, as the reproductive characteristics, growth characteristics and tolerance to pests and pathogens of corn event MZIR098 hybrid were comparable to those of the unmodified control corn hybrid. As feeding damage by corn rootworm larvae is not known to be a major factor restricting the establishment or distribution of corn in Canada, the introduction of this novel trait does not make corn event MZIR098 weedy or invasive of natural habitats. Tolerance to the glufosinate-ammonium herbicide provides a competitive advantage only when this herbicide is used and will not, in and of itself, make the herbicide-tolerant plant weedier or more invasive of natural habitats. Corn event MZIR098 plants growing as volunteers will not be controlled if glufosinate-ammonium herbicides are used as the only weed management tools. However, control of corn event MZIR098 as a volunteer weed in subsequent crops or in fallow ground can be achieved by the use of other classes of herbicides or by mechanical means.

The novel traits have no intended or observed effects on weediness or invasiveness. The CFIA has therefore concluded that corn event MZIR098has no altered weediness or invasiveness potential in Canada compared to currently grown corn varieties.

The CFIA considers the changes in usual agronomic practices that may arise from volunteer plants with novel herbicide tolerances. Similarly, the CFIA considers the potential that continued application of the same herbicide in subsequent rotations may lead to increased selection pressure for herbicide-tolerant weed populations. To address these issues, a herbicide tolerance management plan, which includes integrated weed management strategies, should be implemented. This plan may include a recommendation to rotate or combine weed management products with alternate modes of action and to employ other weed management practices. According to Syngenta Canada Inc., corn event MZIR098 is not intended to be cultivated as an individual event in Canada. Before corn event MZIR098 is cultivated in Canada as an individual event or in combination with other corn events in stacked/pyramided products, Syngenta Canada Inc. must submit a herbicide tolerance management plan to the CFIA.

2. Potential for Gene Flow from Corn Event MZIR098 to Sexually Compatible Plants Whose Hybrid Offspring May Become More Weedy or More Invasive

The CFIA biology document BIO1994-11 – The Biology of Zea mays (L.) (Corn) states that there are no sexually compatible species in Canada that can hybridize with corn. The novel traits (resistance to corn rootworm and tolerance to glufosinate-ammonium) introduced into corn event MZIR098 have no intended effects on corn

reproductive biology.

The CFIA has therefore concluded that gene flow from corn event MZIR098 to sexually compatible relatives is not possible in Canada.

3. Potential for Corn Event MZIR098 to Become a Plant Pest

The resistance to corn rootworm and glufosinate-ammonium tolerance traits introduced into corn event MZIR098 are unrelated to plant pest potential (i.e., the potential for the plant to harbor new or increased populations of pathogens or pests). The mCry3a, eCry3.1Ab and PAT proteins expressed in corn event MZIR098 are not intended or expected to impact the plant pest potential.

Field observations did not indicate differences in the response of the corn event MZIR098 hybrid to biotic stressors including the pathogens Fusarium spp., corn rust, gray leaf spot, northern corn blight, Stewart's disease and common smut, and the insect pests corn earworm, European corn borer, grasshopper, corn rootworm, Japanese beetle and armyworm when compared with the unmodified control corn hybrid.

The CFIA has therefore concluded that corn event MZIR098 does not display any altered plant pest potential compared to currently grown corn varieties.

4. Potential Impact of Corn Event MZIR098 and Its Gene Products on Non-Target Organisms, Including Humans

The glufosinate-ammonium tolerance trait introduced into corn event MZIR098 is unrelated to a potential impact on non-target organisms.

Corn event MZIR098 produces the same eCry3.1Ab and mCry3A proteins as corn events 5307 (DD2013-96) and MIR604 (DD2007-68), respectively. The safety to non-target organisms of the eCry3.1Ab and mCry3A proteins expressed in corn events 5307 and MIR604, respectively, has been previously determined by the CFIA. Thus, data from the dietary toxicity studies submitted in support of the environmental safety of the eCry3.1Ab and mCry3A proteins in corn events 5307 and MIR604 were used to support the environmental safety of the eCry3.1Ab and mCry3A proteins in corn event MZIR098. However, as the protein concentrations and expression patterns in corn event MZIR098 are different from those in corn events 5307 and MIR604, additional analysis was submitted that considered the concentrations of the eCry3.1Ab and mCry3A proteins in the tissues of corn event MZIR098.

The indicator species tested for mCry3A were the flower bug (Orius laevigatus), rove beetle (Aleochara bilineata), pink-spotted lady beetle (Coleomegilla maculata), carabid beetle (Poecilus cupreus), earthworm (Eisenia fetida), honey bee (Apis mellifera), bobwhite quail (Colinus virginianus) and mouse (Mus musculus). The indicator species tested for eCry3.1Ab were the seven-spotted lady beetle (Coccinella septempunctata), pink-spotted lady beetle (Coleomegilla maculata), flower bug (Orius insidiosus), rove beetle (Aleochara bilineata), carabid beetle (Poecilus cupreus), earthworm (Eisenia fetida), honey bee (A. mellifera), bobwhite quail (Colinus virginianus) and mouse (Mus musculus). For all indicator species tested, except the pink-spotted lady beetle for mCry3A, the dietary concentrations evaluated previously (see DD2007-68 and DD2013-96) represent at least the worst-case concentrations that represented non-target organisms could be exposed to from the cultivation of corn event MZIR098 and were adaptable to the current submission.

To further assess the risk to above-ground, predator arthropods, Syngenta Canada Inc. submitted a new study on the effect of mCry3A protein ingestion by Coleomegilla maculata, a lady beetle indicator species. The mCry3A protein was demonstrated to be safe to this indicator species at doses exceeding 2 times the worst-case and 5 times the conservative estimated environmental concentration of the mCry3A protein in the diet of non-target arthropods feeding on corn event MZIR098 pollen or exposed to corn event MZIR098 via their prey. Thus, the data suggest that cultivation of corn event MZIR098 will pose negligible risk to above-ground predator arthropods.

Detailed characterization of the mCry3A, eCry3.1Ab and PAT proteins expressed in corn event MZIR098 led to the conclusion that these proteins do not display any characteristic of a potential putative mammalian toxin or allergen (see Section V, part 2: Potential Impact of Corn Event MZIR098 on Animal Health and Human Safety as it Relates to the Potential Transfer of Residues into Foods of Animal Origin and Worker/Bystander Exposure to the Feed).

Therefore, no negative impacts resulting from exposure of organisms to the mCry3A, eCry3.1Ab and PAT proteins expressed in corn event MZIR098 are expected.

Composition analyses showed that the levels of key nutrients and anti-nutrients in grain and forage from corn event MZIR098 are comparable to those in the unmodified control corn hybrid (see Section V, part 1: Potential Impact of Corn Event MZIR098 on Livestock Nutrition). Therefore, it is very unlikely that the introduction of the novel traits may have caused unintended changes to the composition of corn event MZIR098 tissues that would negatively impact organisms interacting with corn event MZIR098.

No new or increased populations of pathogens or pests were observed in corn event MZIR098 hybrid when qualitatively compared to the unmodified control corn hybrid (see Section IV, part 3: Potential for Corn Event MZIR098 to Become a Plant Pest).

Collectively, these information elements indicate that the interactions between corn event MZIR098 and the populations of animals and microorganisms interacting with corn crops will be similar to currently grown corn varieties.

Based on the above information, the CFIA has concluded that the unconfined release of corn event MZIR098 in Canada will not result in altered impacts on non-target organisms, including humans, compared to currently grown corn varieties.

5. Potential Impact of Corn Event MZIR098 on Biodiversity

Corn event MZIR098 expresses no novel phenotypic characteristics that would extend its geographic range beyond the current 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. Corn event MZIR098 is unlikely to cause adverse effects on non-target organisms and does not display increased weediness, invasiveness or plant pest potential. It is therefore unlikely that corn event MZIR098 will have any direct effects on biodiversity, in comparison to the effects that would be expected from the cultivation of the corn varieties that are currently grown in Canada.

Corn event MZIR098 has resistance to corn rootworm and tolerance to the herbicide glufosinate-ammonium. At present, crop rotation, the use of corn rootworm resistant corn varieties and chemical insecticide seed treatments are common practices to manage corn rootworms in Canada. The change in local corn rootworm populations as a result of the release of corn event MZIR098 will not present a major change from existing agricultural practices. The use of the glufosinate-ammonium herbicide in cropping systems has the intended effect of reducing local weed populations within agro-ecosystems. This may result in a reduction in local weed species biodiversity, and may have effects on other trophic levels that utilize these weed species. It must be noted, however, that the goal of reduction in weed biodiversity in agricultural fields is not unique to the use of plants with novel traits, corn event MZIR098 or the cultivation of corn. It is therefore unlikely that corn event MZIR098 will have any indirect effects on biodiversity, in comparison to the effects that would be expected from the cultivation of currently grown corn varieties.

The CFIA has concluded that the introduced genes and their corresponding novel traits do not confer to corn event MZIR098 any characteristic that would result in unintended environmental effects following unconfined release. The CFIA has therefore concluded that the potential impact on biodiversity of corn event MZIR098 is unlikely to be different from that of the corn varieties that are currently grown in Canada.

6. Potential for Development of Insect Resistance to Corn Event MZIR098

To minimize the likelihood of the development of insect resistance to a plant 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 control measures including conventional chemical insecticides. Therefore, it is reasonable to expect that resistance to the insecticidal proteins expressed in corn event MZIR098 may develop. An insect resistance management plan designed to reduce or delay corn rootworm resistance to the eCry3.1Ab and mCry3A proteins must be implemented for the cultivation of corn event MZIR098 corn in Canada. Before corn event MZIR098 is cultivated in Canada as an individual event or in combination with other corn events in stacked/pyramided products, Syngenta must submit an insect resistance management plan to the CFIA.

V. Criteria for the Livestock Feed Assessment

The AFD considered nutrient and anti-nutrient profiles; the safety of feed ingredients derived from corn event MZIR098, including the presence of gene products, residues and metabolites in terms of animal health and human safety as it relates to the potential transfer of residues into foods of animal origin and worker/bystander exposure to the feed; and whether feeds derived from corn event MZIR098 meet the definitions and requirements of feeds as listed in Schedule IV of the Feeds Regulations.

1. Potential Impact of Corn Event MZIR098 on Livestock Nutrition

Nutrient and anti-nutrient composition

The nutritional equivalence of corn event MZIR098 hybrid (sprayed and unsprayed plants with glufosinate-ammonium) to those of the unsprayed, unmodified control corn hybrid and six unsprayed, reference corn varieties was determined from 8 replicated filed trials in the US during the 2013 growing season. Forage and grain samples were analysed for moisture, ash, protein, crude fat, carbohydrates, calcium, phosphorus, acid detergent fibre (ADF) and neutral detergent fibre (NDF). Grain samples were further analysed for amino acids, fatty acids, vitamins, minerals, total dietary fiber (TDF), starch, secondary metabolites (p-coumaric acid, ferulic acid, furfural and inositol) and anti-nutrients (phytic acid, raffinose and trypsin inhibitor) as recommended by the OECD consensus document for new varieties of corn (OECD, 2002).

Composition data was analyzed statistically using a mixed model analysis of variance and statistical differences among treatments were identified and assessed ($P<0.05$). The biological relevance of any significant difference among corn varieties was assessed by comparing the observed values to the range of values observed for the reference corn varieties grown in the trials, and in the published scientific literature (ILSI, 2014).

No statistically significant differences were observed between forage from corn event MZIR098 hybrid and the unmodified control corn hybrid for protein, fat, carbohydrates, ash, ADF, NDF, calcium and phosphorus.

No statistically significant differences were observed between grain samples from corn event MZIR098 hybrid (unsprayed) and the unmodified control corn hybrid for protein, fat, ash, carbohydrates, starch, ADF and NDF. Statistically significant differences were observed between the corn event MZIR098 hybrid (sprayed) and the unmodified control corn hybrid for NDF and starch, but these differences were not biologically significant as all means were within the range of the values observed for the reference corn varieties grown in the trials and in the published scientific literature (ILSI, 2014). Except for calcium, potassium and copper, no statistically significant differences were found between the corn event MZIR098 hybrid and the unmodified control corn hybrid for minerals. Means levels of calcium, potassium and copper were within the range of the values observed in the reference corn varieties grown in the trials and the published scientific literature (ILSI, 2014). Except for vitamins A, and E, no

statistically significant differences were observed between grain samples from the corn event MZIR098 hybrid and the unmodified control corn hybrid vitamins. The differences observed for vitamins A and E are not biologically significant as means levels were within the range of the values observed for reference corn varieties grown in the trials and the published scientific literature (ILSI, 2014). Except for lysine, no statistically significant differences were found between the novel corn event MZIR098 hybrid (unsprayed) and the unmodified control corn hybrid for the amino acids. Means level of lysine was within the range of the values observed in the reference corn varieties grown in the trials and the published scientific literature (ILSI, 2014). No statistically significant differences were observed between grain samples from corn event MZIR098 hybrid and the unmodified control corn hybrid for palmitic, palmitoleic, linolenic, eicosenoic and behenic acids. Statistically significant differences were observed between the corn event MZIR098 (sprayed) and the unmodified control corn hybrid for heptadecanoic, stearic, oleic, linoleic and arachidic acids. However, all the means were within the range of the values observed in the reference corn varieties grown in the trials and the published scientific literature (ILSI, 2014). No statistically significant differences were observed between grain samples from corn event MZIR098 hybrid (sprayed and unsprayed) and the unmodified control corn hybrid for secondary metabolites (p-Coumaric, ferulic acid, furfural, and inositol) and anti-nutrients (phytic acid, raffinose and trypsin inhibitor).

Conclusion

It was concluded, based on the evidence provided by Syngenta Canada Inc. that the nutritional composition of the corn event MZIR098 hybrid is similar to that of unmodified control corn hybrids grown in the same trials and to that reported for other corns in the published scientific literature. Feed ingredients derived from corn event MZIR098 are considered to meet present ingredient definitions for corn in Schedule IV of the Feeds Regulations.

2. Potential Impact of Corn Event MZIR098 on Animal Health and Human Safety as it Relates to the Potential Transfer of Residues into Foods of Animal Origin and Worker/Bystander Exposure to the Feed

Corn event MZIR098 is active against western corn rootworm (Diabrotica virgifera virgifera) and other related coleopteran pests of corn due to production of the mCry3A and eCry3.1Ab proteins, and tolerant to glufosinate-ammonium herbicides due to production of the PAT protein.

The assessment of corn event MZIR098 used the weight of evidence approach to evaluate the impact of the following potential hazards on the safety of feed ingredients derived from this event:

- The novel mCry3A protein
- The novel eCry3.1Ab protein
- The novel PAT protein
- The chemical pesticide residue profile

mCry3A Protein

To obtain sufficient quantities of mCry3A protein for evaluation of environmental and feed safety, it was necessary to express the mcry3A gene in a microbial production system. Equivalency was demonstrated among the mCry3A protein expressed in corn event MZIR098, the mCry3A protein expressed in corn event MIR604 (DD2007-68) as well as the mCry3A protein produced in the E. coli production system used in safety studies of corn event MIR604. Equivalency was established by comparing their molecular weights, immunoreactivities, glycosylation status, N- and C- terminal amino-acid sequence, and/or peptide mass spectrometry. Based on the results, the proteins were found to be equivalent. Demonstration of the equivalence among the mCry3A protein produced in corn event MZIR098, in corn event MIR604 and in the E. coli-production system allows studies with E. coli-produced mCry3A protein and studies conducted in support of safety of corn event MIR604 to be used in support of safety of corn event MZIR098.

The potential allergenicity and toxicity of the mCry3A protein to livestock were evaluated. With respect to its potential allergenicity, no single experimental method yields decisive evidence, thus a weight-of-evidence approach was taken, taking into account information obtained with various test methods. The source of the mcry3A gene, B. thuringiensis subsp. tenebrionis, is not commonly associated with allergenicity and a bioinformatics evaluation of the mCry3A protein amino acid sequences confirmed the lack of relevant similarities between this protein and known allergens. Unlike many allergens, the mCry3A protein produced inan E. coli production system, previously evaluated in support of safety of corn event MIR604, was shown experimentally to be rapidly degraded in simulated gastric fluid and to be denatured by heat, and this protein expressed in corn event MZIR098 was shown experimentally to be non-glycosylated. The weight of evidence thus indicates that the mCry3A protein is unlikely to be allergenic.

In terms of the potential toxicity to livestock, the mCry3A protein lacks a mode of action to suggest that it is intrinsically toxic to livestock and a bioinformatics evaluation of its protein amino acid sequence confirmed the lack of relevant similarities between the mCry3A protein and known toxins. The amino acid sequence of the mCry3A protein produced in corn event MZIR098 is identical to that of the mCry3A protein in the previously authorized MIR604 (DD2007-68). Additionally, data submitted in support of corn event MIR604 safety demonstrated no adverse effects when the E. coli-produced mCry3A protein was ingested by mice at a dose of approximately 2377 mg/kg body weight. Further, the livestock exposure to the mCry3A protein is expected to be negligible as this protein is expressed at very low levels in corn event MZIR098, is rapidly degraded under conditions which simulate the mammalian digestive tract, and is unstable under heating conditions expected to be encountered during processing of some corn products. The weight of evidence thus indicates that the mCry3A protein is unlikely to be toxic to livestock.

eCry3.1Ab Protein

To obtain sufficient quantities of eCry3.1Ab protein for evaluation of environmental and feed safety, it was necessary to express the ecry3.1ab gene in a microbial production system.

Equivalency was demonstrated among the eCry3.1Ab protein expressed in corn event MZIR098, the eCry3.1Ab protein expressed in corn event 5307 (DD2013-96) as well as the eCry3.1Ab protein produced in the E. coli production system used in safety studies of corn event 5307. Equivalency was established by comparing their molecular weights, immunoreactivity, glycosylation status, N- and C- terminal amino acid sequence, and/or peptide mass spectrometry. Based on the results, the proteins were found to be equivalent. Demonstration of the equivalence among the eCry3.1Ab proteins produced in corn event MZIR098, in corn event 5307 and in the E. coli-production system allows studies with E. coli-produced eCry3.1Ab protein and studies conducted in support of safety of corn event 5307 to be used in support of safety of corn event MZIR098.

The potential allergenicity and toxicity of the eCry3.1Ab protein to livestock were evaluated. With respect to its potential allergenicity, no single experimental method yields decisive evidence, thus a weight-of-evidence approach was taken, taking into account information obtained with various test methods. The source of the ecry3A.1Ab gene, B. thuringiensis subsp. kurstaki, is not commonly associated with allergenicity and a bioinformatics evaluation of the eCry3.1Ab protein amino acid sequences confirmed the lack of relevant similarities between this protein and known allergens. Unlike many allergens, the eCry3.1Ab protein produced in an E. coli production system, evaluated in support of safety of corn event 5307, was shown experimentally to be rapidly degraded in simulated gastric fluid and to be denatured by heat, and this protein expressed in corn event MZIR098 was shown experimentally to be non-glycosylated. The weight of evidence thus indicates that the eCry3.1Ab protein is unlikely to be allergenic.

In terms of the potential toxicity to livestock, the eCry3.1Ab protein lacks a mode of action to suggest that it is intrinsically toxic to livestock and a bioinformatics evaluation of its protein amino acid sequence confirmed the lack of relevant similarities between the eCry3.1Ab protein and known toxins. The amino acid sequence of the eCry3.1Ab protein produced in corn event MZIR098 is identical to that of eCry3.1Ab protein in the previously

authorized corn event 5307 (DD2013-96). Additionally, data submitted in support of corn event 5307 safety demonstrated no adverse effects when the E. coli-produced eCry3.1Ab protein was ingested by mice at a dose of approximately 2000 mg/kg body weight. Further, the livestock exposure to the eCry3.1Ab protein is expected to be negligible as this protein is expressed at very low levels in corn event MZIR098, is rapidly degraded under conditions which simulate the mammalian digestive tract, and is unstable under heating conditions expected to be encountered during processing of some corn products. The weight of evidence thus indicates that the eCry3.1Ab protein is unlikely to be toxic to livestock.

PAT Protein

To obtain sufficient quantities of PAT protein for evaluation of environmental and feed safety, it was necessary to express the pat gene in a microbial production system. Equivalency was demonstrated among the PAT protein expressed in corn event MZIR098, the PAT protein expressed in corn event MZHG0JG (DD2016-116), the PAT protein expressed in corn event Bt11 (DD1996-12), as well as the PAT protein produced in the E. coli production system. Equivalency was established by comparing their molecular weights, immunoreactivity, glycosylation status, and peptide mass spectrometry. Based on the results, the proteins were found to be equivalent. Demonstration of the equivalence among the PAT proteins allows studies with E. coli-produced PAT protein and the studies conducted in support of safety of corn event MZHG0JG to be used in support of safety of corn event MZIR098.

The potential allergenicity and toxicity of the PAT protein to livestock were evaluated. With respect to its potential allergenicity, no single experimental method yields decisive evidence, thus a weight-of-evidence approach was taken, taking into account information obtained with various test methods. The source of the pat gene, S. viridochromogenes, is not commonly associated with allergenicity and a bioinformatics evaluation of the PAT protein amino acid sequence confirmed the lack of relevant similarities between this protein and known allergens. Unlike many allergens, the PAT protein produced inan E. coli production system, evaluated in support of safety of corn event MZHG0JG, was shown experimentally to be rapidly degraded in simulated gastric and intestinal fluid and to be denatured by heat, and this protein expressed in corn event MZIR098 was shown experimentally to be non-glycosylated. The weight of evidence thus indicates that the PAT protein is unlikely to be allergenic.

In terms of the potential toxicity to livestock, the PAT protein lacks a mode of action to suggest that it is intrinsically toxic to livestock and a bioinformatics evaluation of its protein amino acid sequence confirmed the lack of relevant similarities between the PAT protein and known toxins. The amino acid sequence of the PAT protein produced in corn event MZIR098 was demonstrated to be identical to a previously assessed PAT protein. The livestock exposure to the PAT protein is expected to be negligible as this protein is expressed at very low levels in corn event MZIR098, is rapidly degraded under conditions which simulate the mammalian digestive tract, and is unstable under heating conditions expected to be encountered during processing of some corn products. The weight of evidence thus indicates that the PAT protein is unlikely to be toxic to livestock.

Chemical pesticide residue profile

The herbicide residues and metabolites in feed commodities from corn event MZIR098, following application of herbicides, were also evaluated as part of the feed safety assessment. It was determined that potential glufosinate-ammonium residues and metabolites in livestock commodities derived from corn event MZIR098 and its products would not be expected to present levels of safety concern to livestock, nor to humans via the potential transfer into foods of animal origin.

Conclusion:

It was concluded, based on the evidence provided by Syngenta Canada Inc., that the mCry3A and eCry3.1Ab proteins-based insect resistance and the PAT protein-based herbicide tolerance traits will not confer to corn event MZIR098 any characteristic that would raise concerns regarding the safety of corn event MZIR098. Feed ingredient

(s) from corn event MZIR098 are considered to meet present ingredient definitions for corn in the Feeds Regulations and as such are approved for use as livestock feed in Canada.

VI. New Information Requirements

If at any time, Syngenta Canada Inc. becomes aware of any new information regarding risk to the environment, livestock or human health, which could result from the unconfined environmental release or livestock feed use of corn event MZIR098 or lines derived from it, Syngenta Canada Inc. is required to immediately provide such information to the CFIA. On the basis of such new information, the CFIA will re-evaluate the potential impact of corn event MZIR098 on the environment, livestock and human health and may re-evaluate its decision with respect to the livestock feed use and unconfined environmental release authorizations of corn event MZIR098.

VII. Regulatory Decision

Based on the review of the data and information submitted by Syngenta Canada Inc. and input from other relevant scientific sources, the Plant and Biotechnology Risk Assessment Unit of the Plant Health Science Directorate, CFIA, has concluded that the unconfined environmental release of corn event MZIR098 does not present altered environmental risk when compared to corn varieties that are currently grown in Canada.

Based on the review of the data and information submitted by Syngenta Canada Inc. and input from other relevant scientific sources, the Animal Feed Division of the Animal Health Directorate, CFIA, has concluded that the novel Cry3.1Ab and mCry3A proteins, and the novel PAT protein-based herbicide tolerance trait will not confer to corn event MZIR098 any characteristic that would raise any concerns regarding the safety or nutrition of corn event MZIR098. Grain corn, its by-products and corn oil are currently listed in Schedule IV of the Feeds Regulations and are, therefore authorized for use in livestock feeds in Canada. Corn event MZIR098 has been found to be as safe as and as nutritious as currently and historically grown corn varieties. Corn event MZIR098 and its products are considered to meet present ingredient definitions and are authorized for use as livestock feed ingredients in Canada.

Unconfined release into the environment and use as livestock feed of corn event MZIR098 is therefore authorized by the Plant Biosafety Office of the Plant Health and Biosecurity Directorate and the Animal Feed Division of the Animal Health Directorate, respectively, as of August 9th, 2016. Any corn lines derived from the corn event MZIR098 may also be released into the environment and used as livestock feed, provided that:

(i) no inter-specific crosses are performed
(ii) the intended use (s) are similar,
(iii) it is known based on characterization that these plants do not display any additional novel traits and are substantially equivalent to corn varieties that are currently grown and permitted to be used as livestock feed in Canada, in terms of their potential environmental impact and livestock feed safety and nutrition, and
(iv) the novel genes are expressed at levels similar to that of the authorized line.

With respect to its unconfined release into the environment, cultivation of corn event MZIR098 is subject to insect resistance management requirements.

Additionally, with respect to its unconfined release into the environment, an appropriate herbicide tolerance management plan should be implemented.

Before corn event MZIR098 is cultivated in Canada as an individual event or in combination with other corn events in stacked/pyramided products, Syngenta Canada Inc. must submit a herbicide tolerance management plan to the CFIA.

Corn event MZIR098 is subject to the same phytosanitary import requirements as unmodified corn varieties. Corn event MZIR098 must also meet the requirements of other Canadian legislation, including but not limited to the requirements set out in the Food & Drugs Act and the Pest Control Products Act.

Please refer to Health Canada's Decisions on Novel Foods for a description of the food safety assessment of corn event MZIR098.

Date modified:

2018-11-23