Canada

Canadian Food Inspection Agency

Decision Document DD2016-116 Determination of the Safety of Syngenta Canada Inc.'s Corn (Zea mays L.) Event MZHG0JG

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 herbicide-tolerant corn event MZHG0JG. 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 MZHG0JG 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 May 16th, 2016. Any corn lines derived from corn event MZHG0JG 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 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 a level similar to that of the authorized line.

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

Corn event MZHG0JG is subject to the same phytosanitary import requirements as unmodified corn varieties. Corn event MZHG0JG 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.

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I. Brief Identification of the Modified Plant

Designation of the Modified Plant:	Corn event MZHG0JG, OECD Unique Identifier SYN-ØØØJG-2
Applicant:	Syngenta Canada Inc.
Plant Species:	Corn (Zea mays L.)
Novel Traits:	Tolerance to glyphosate and glufosinate-ammonium herbicides
Trait Introduction Method:	Agrobacterium-mediated transformation

Intended Use of the Modified Plant:	Corn event MZHG0JG is intended for human consumption and livestock feed uses. Corn event MZHG0JG 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 tolerant to the herbicides glyphosate and glufosinate-ammonium. Corn event MZHG0JG was developed using recombinant deoxyribonucleic acid (rDNA) technology, resulting in the introduction of a modified 5-enolpyruvylshikimate-2-phosphate synthase (mepsps) gene and a phosphinothricin N-acetyltransferase (pat) gene. The mepsps gene was originally isolated from Z. mays, mutated by site-directed mutagenesis and encodes a modified 5-enolpyruvylshikimate-2-phosphate synthase (mEPSPS) protein. The mEPSPS protein is not inhibited by glyphosate, thus conferring tolerance to the herbicide. 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.

Syngenta Canada Inc. has provided information on the identity of corn event MZHG0JG; 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 the potential allergenicity of the novel proteins to humans and livestock.

A hybrid of corn event MZHG0JG (hereafter referred to as the corn event MZHG0JG hybrid) was field tested in the United States (US) at eight 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. A hybrid of an unmodified control corn variety (hereafter referred to as the the unmodified control corn hybrid), which shares similar genetic background as corn event MZHG0JG, was included in the trials to act as a comparator for the corn event MZHG0JG hybrid. 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 MZHG0JG 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 from the reference corn hybrids.

Nutritional components of grain and forage from the corn event MZHG0JG 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 ranges established from 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 the environmental safety of PNTs, as described in Directive 94-08 – Assessment Criteria for Determining Environmental Safety of Plants with Novel Traits. The PBRA Unit has considered:

- the potential for corn event MZHG0JG to become a weed of agriculture or to be invasive of natural habitats;
- the potential for gene flow from corn event MZHG0JG to sexually compatible plants whose hybrid offspring may become more weedy or more invasive;
- the potential for corn event MZHG0JG to become a plant pest;
- the potential impact of corn event MZHG0JG and its gene products on non-target organisms, including humans; and

- the potential impact of corn event MZHG0JG 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 of corn event MZHG0JG, and similarities and differences between corn event MZHG0JG and unmodified control corn varieties relative to the safety and nutrition of feed ingredients derived from corn event MZHG0JG for their intended purpose, including:

- the potential impact of corn event MZHG0JG on livestock nutrition; and
- the potential impact of corn event MZHG0JG 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.

The AFD has also considered whether feeds derived from corn event MZHG0JG 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 MZHG0JG was developed through Agrobacterium-mediated transformation of immature Z. mays embryos, harvested from a proprietary maize line. Transformation was accomplished using a transformation plasmid vector that included a single T-DNA, containing the mepsps and pat expression cassettes. Following cultivation with Agrobacterium, embryonic calli, derived from transformed embryos, were transferred to a selection medium containing glufosinate-ammonium to select for cells containing the T-DNA. Plants containing the mepsps and pat genes were confirmed by molecular analysis. Corn event MZHG0JG was identified as a successful transformant and was chosen for further development.

2. Tolerance to Glyphosate Herbicide

EPSPS is an enzyme involved in the plant shikimic acid metabolic pathway, which is essential for the production of aromatic amino acids. Glyphosate disrupts the shikimate pathway by binding to and inhibiting the EPSPS enzyme. This inhibition results in reduced aromatic amino acid production, growth suppression and eventually plant death. Corn event MZHG0JG was developed to be tolerant to the herbicide glyphosate by incorporation of the mepsps gene derived from maize (Z. mays), which encodes a modified EPSPS (mEPSPS) containing two amino acid substitutions that reduce the enzyme's affinity for glyphosate.

The expression of the mEPSPS protein in corn event MZHG0JG is driven by a constitutive promoter. Samples of corn tissues from plants sprayed with glyphosate and glufosinate-ammonium were collected from four field trial sites in the US. The average mEPSPS protein levels across all sites, expressed in micrograms of protein per gram dry weight tissue (µg/g dwt), as evaluated by enzyme-linked immunosorbent assay (ELISA), were as follows: 1103 ±798 µg/g dwt in leaf, 322 ±91.96 µg/g dwt in root, 298 ± 220 µg/g dwt in whole plant, below limits of quantification in pollen and 61.14 ± 15.49 µg/g dwt in kernel.

The potential allergenicity and toxicity of the mEPSPS protein were evaluated. The weight of evidence indicates that the mEPSPS protein is unlikely to be allergenic, since the source of the mepsps gene, Z. mays, is not known to produces allergens, and since the mEPSPS protein amino acid sequence lacks relevant similarities to known allergens. Furthermore, the Escherichia coli (E. coli)-produced mEPSPS protein was shown experimentally to be rapidly degraded in simulated gastric fluid and simulated intestinal fluid, which is unlike many allergens. Finally,

unlike many allergens, corn event MZHG0JG-produced mEPSPS protein was shown experimentally to be non-glycosylated. It was also concluded that the mEPSPS 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 since the mEPSPS protein amino-acid sequence lacks relevant similarities to known toxins.

For a more detailed discussion of the potential allergenicity and toxicity of the mEPSPS protein, see Section V, part 2: Potential Impact of Corn Event MZHG0JG 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

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 MZHG0JG was developed to be tolerant to the herbicide glufosinate-ammonium 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. The pat gene was derived from S. viridochromogenes, a gram-positive soil bacterium and the PAT protein produced in corn event MZHG0JG is identical to the native enzyme. Introduction of the pat gene into corn event MZHG0JG confers commercial-level tolerance to glufosinate-ammonium herbicide.

The pat gene expression in corn event MZHG0JG is driven by a constitutive promoter. Samples of corn tissues from plants sprayed with glyphosate and glufosinate-ammonium were collected from four field trial sites in the US. The average PAT protein levels across all sites, as evaluated by ELISA were 2.26 ±2.02 µg/g dwt in leaf, 1.29 ±0.9 µg/g dwt in root, and below the limits of quantification in whole plant, pollen, and kernel.

The PAT protein expressed in corn event MZHG0JG is identical to the PAT protein found in several commercial corn products, including Liberty Link corn (DD98-22) and corn event Bt11 (DD1996-12). The PAT protein has a history of safe use in Canada, and as such is not expected to be toxic or allergenic.

The potential allergenicity and toxicity of the PAT protein expressed in corn event MZHG0JG were evaluated further. 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 known to produce allergens and the PAT protein amino acid sequence lacks relevant similarities to known allergens. Unlike many allergens, the E. coli-produced PAT protein was shown experimentally to be rapidly degraded in simulated gastric fluid and simulated intestinal fluid. Finally, unlike many allergens, corn event MZHG0JG PAT protein 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 MZHG0JG on Animal Health and Human Safety as it Relates to the Potential Transfer of Residue 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 MZHG0JG contains one intact copy of the mepsps 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 MZHG0JG.

The stability of the insert within corn event MZHG0JG was studied over five generations by Southern blot analysis. This study clearly demonstrated that sequential generations of corn event MZHG0JG stably inherited the insert.

The inheritance pattern of the insert was determined by real-time PCR analysis over three segregating generations of corn event MZHG0JG. The results indicated that the insert segregates according to the Mendelian rules of inheritance for a single genetic locus.

IV. Criteria for the Environmental Assessment

1. Potential for Corn Event MZHG0JG 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 MZHG0JG 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 MZHG0JG. As previously mentioned, a corn event MZHG0JG hybrid was tested in the US at eight field 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 MZHG0JG 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 a range of comparative values for each characteristic that are representative of currently grown corn varieties. 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 statistically significant differences were observed between the corn event MZHG0JG hybrid and the unmodified control corn hybrid in ear height, plant height, stay green, grain moisture or test weight. The mean grain yield and early stand count (pre-thinning) of corn event MZHG0JG hybrid were statistically significantly lower than the unmodified control corn hybrid, but within the ranges established from the reference corn hybrids. The numerical difference for early stand count between corn event MZHG0JG hybrid and the unmodified control corn hybrid was small. A reduction in early stand count is not associated with an increase in weediness potential and can be influenced by weather, planter issues or insect feeding. The numerical difference for grain yield between corn event MZHG0JG hybrid and the unmodified control corn hybrid was small. A reduction in grain yield is not associated with an increase in weediness potential. Therefore, these differences were not considered to be biologically meaningful. 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 MZHG0JG 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 MZHG0JG to currently grown corn varieties.

Syngenta Canada Inc. provided information on the dormancy and germination of corn event MZHG0JG seed under six different temperature regimes. Seed germination characteristics were evaluated, including percent germinated seed (normal and/or abnormal), percent hard seed, percent dead seed and percent dormant seed. The corn event MZHG0JG hybrid was compared to an unmodified control corn hybrid with a similar genetic background. Three reference corn hybrids were included to provide a range of comparative values for each germination characteristic.

Statistically significant differences were observed between the corn event MZHG0JG hybrid and the unmodified control corn hybrid at two temperature regimes for the percent of germinated seed (normal). However, the values for the corn event MZHG0JG hybrid were only slightly lower than that of the unmodified control corn hybrid and were within the range of values established from the reference corn hybrids. Therefore these instances of statistically significant differences are not considered biologically meaningful. There were no statistical significant differences in percent germinated seed (abnormal) (evaluated under two of the temperature regimes), percent dead seed or percent dormant seed between the corn event MZHG0JG hybrid and the unmodified control corn hybrid. Moreover, a lack of hard seed indicated that corn event MZHG0JG 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 the corn event MZHG0JG hybrid to abiotic stressors was observed at 8 sites in 2013. The stressors observed included drought, heat stress, nutrient deficiency and wind. No consistent trend in increased or decreased susceptibility to these abiotic stressors was observed in the corn event MZHG0JG hybrid compared to the unmodified control corn hybrid.

The susceptibility of the corn event MZHG0JG hybrid to corn pests and pathogens was observed at eight sites in 2013. The stressors observed included corn earworm, Fusarium spp., corn rust, corn borer, grasshopper, 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 MZHG0JG hybrid compared to the unmodified control hybrid.

No competitive advantage was conferred to plants of corn event MZHG0JG, other than that conferred by tolerance to the glyphosate and glufosinate-ammonium herbicides, as the reproductive characteristics, growth characteristics and tolerance to pests and pathogens of corn event MZHG0JG were comparable to those of the unmodified control corn hybrid. Tolerance to the glyphosate and glufosinate-ammonium herbicides provides a competitive advantage only when one, or both, of these herbicides are used and will not, in and of itself, make the herbicide-tolerant plant weedier or more invasive of natural habitats. Corn event MZHG0JG plants growing as volunteers will not be controlled if the glyphosate and glufosinate-ammonium herbicides are the only weed control tools used. However, control of corn event MZHG0J Gas a volunteer weed in subsequent crops or in fallow ground can be achieved using other classes of herbicides or mechanical means.

The novel traits have no intended or observed effects on weediness or invasiveness. The CFIA has therefore concluded that corn event MZHG0JG has 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. In order 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 control products with alternate modes of action and to employ other weed control practices.

Syngenta Canada Inc. previously submitted a herbicide tolerance management plan to the CFIA, which was determined to be satisfactory when evaluated by the PBRA Unit.

Syngenta Canada Inc. will make this herbicide tolerance management plan readily available to growers and agriculture extension personnel, in both private and public sectors, to promote careful management practices for corn event MZHG0JG. Syngenta Canada Inc. will provide an efficient mechanism for growers to report agronomic problems to the company, which will facilitate the ongoing monitoring of corn event MZHG0JG. Syngenta Canada Inc. will monitor grower implementation to determine the effectiveness of the herbicide tolerance management plan and make any changes to the plan as appropriate.

2. Potential for Gene Flow from Corn Event MZHG0JG 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 glyphosate and glufosinate-ammonium traits introduced into corn event MZHG0JG have no intended effects on corn reproductive biology.

The CFIA has therefore concluded that gene flow from corn event MZHG0JG to sexually compatible relatives is not possible in Canada.

3. Potential for Corn Event MZHG0JG to Become a Plant Pest

The glyphosate and glufosinate-ammonium tolerance traits introduced into corn event MZHG0J Gare unrelated to plant pest potential (i.e. the potential for the plant to harbor new or increased populations of pathogens or pests). The mEPSPS and PAT proteins expressed in corn event MZHG0JG are not intended or expected to impact the plant pest potential. The mEPSPS and PAT proteins produced in corn event MZHG0JG have been present in commercial corn varieties for almost two decades, both as single events and in combination through trait stacking with conventional breeding. No changes in plant pest potential have been reported since the commercialization of these products.

Field observations did not indicate differences in the response of corn event MZHG0JG to biotic stressors including corn earworm, Fusarium spp., corn rust, corn borer, grasshopper, rootworm, gray leaf spot, northern corn blight, Stewart's disease and common smut when compared with the unmodified control corn hybrid.

The CFIA has therefore concluded that corn event MZHG0JG does not display any altered plant pest potential compared to currently grown corn varieties.

4. Potential Impact of Corn Event MZHG0JG and Its Gene Products on Non-Target Organisms, Including Humans

The glyphosate and glufosinate-ammonium tolerance traits introduced into corn event MZHG0JG are unrelated to a potential impact on non-target organisms.

Detailed characterization of the mEPSPS and PAT proteins expressed in corn event MZHG0JG led to the conclusion that these proteins do not display any characteristic of a potential toxin or allergen (see Section V, part 2: Potential Impact of Corn Event MZHG0JG 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 mEPSPS and PAT proteins expressed in corn event MZHG0JG are expected.

Composition analyses showed that the levels of key nutrients and anti-nutrients in grain and forage from corn event MZHG0JG are comparable to those in the unmodified control corn hybrid (see Section V, part 1: Potential Impact of Corn Event MZHG0JG 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 MZHG0JG tissues that would negatively impact organisms interacting with corn event MZHG0JG.

The glyphosate and glufosinate-ammonium tolerance traits introduced into corn event MZHG0JGare unrelated to plant pest potential (see Section IV, part 3: Potential for Corn Event MZHG0JG to Become a Plant Pest).

Collectively, these information elements indicate that the interactions between corn event MZHG0JG 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 MZHG0JG 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 MZHG0JG on Biodiversity

Corn event MZHG0JG expresses no novel phenotypic characteristics that would extend its geographic range beyond the current range of corn production in Canada. Since corn has no sexually compatible 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 MZHG0JG 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 MZHG0JG 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 MZHG0JG has tolerance to the herbicides glyphosate and glufosinate-ammonium. The use of these herbicides 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 PNTs, corn event MZHG0JG or the cultivation of corn. It is therefore unlikely that corn event MZHG0JG will have any indirect effects on biodiversity, in comparison to the effects that would be expected from cultivation of the corn varieties that are currently grown in Canada.

The CFIA has concluded that the introduced genes and their corresponding novel traits do not confer to corn event MZHG0JG 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 MZHG0JG is unlikely to be different from that of the corn varieties that are currently grown in Canada.

V. Criteria for the Livestock Feed Assessment

The AFD considered nutrient and anti-nutrient profiles; the safety of feed ingredients derived from corn event MZHG0JG, 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 MZHG0JG meet the definitions and requirements of feeds as listed in Schedule IV of the Feeds Regulations.

1. Potential Impact of Corn Event MZHG0JG on Livestock Nutrition

Nutrient and anti-nutrient composition

The nutritional equivalence of the corn event MZHG0JG hybrid (sprayed and unsprayed plants with glyphosate and glufosinate-ammonium) to those of the unsprayed, unmodified control corn hybrid and six unsprayed, reference corn varieties was determined from eight replicated field trails in the US during the 2013 growing season. Grain and Forage samples were analyzed for moisture, ash, protein, crude fat, carbohydrates (by calculation), 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 statistically significant difference among corn varieties was assessed by comparing the observed mean values to the range of the 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 MZHG0JG hybrid and the unmodified control corn hybrid for fat, ash, ADF and NDF. Statistically significant effect was found between corn event MZHG0JG hybrid and the unmodified control corn hybrid for carbohydrate. Additionally, statistically significant differences were found for protein and carbohydrates in corn event MZHG0JG hybrid (sprayed) and the unmodified control corn hybrid. However, these differences were not biologically significant as all means were within the range of the values observed in the reference corn varieties grown in the trials and in the published literature (ILSI, 2014). No statistically significant difference was observed between forage from corn event MZHG0JG hybrid (unsprayed) and the unmodified control corn hybrid for calcium. Statistically significant difference was found between corn event MZHG0JG hybrid and unmodified control corn hybrid for phosphorus. However, the difference was not biologically significant as the mean was within the range of values observed in the reference corn varieties grown in the trials and in the published literature (ILSI, 2014).

No statistically significant differences were observed between grain samples from corn event MZHG0JG hybrid and the unmodified control corn hybrid for protein, fat, ash, carbohydrates, ADF and starch. Statistically significant effect was found between corn event MZHG0JG hybrid (unsprayed) and the unmodified control corn hybrid for NDF. However, the difference was not biologically significant as the mean was within the range of the values observed in the reference corn varieties grown in the trials and the published literature (ILSI, 2014). Except for copper and iron, no statistically significant differences were found between the corn event MZHG0JG hybrid (unsprayed) and the unmodified control corn hybrid for mineral. Additionally, a statistically significant difference was found for potassium between the corn event MZHG0JG hybrid (sprayed) and the unmodified control corn hybrid. However, all means were within the range of the values observed in the reference corn grown in the trials and in the published literature (ILSI, 2014).

Except for vitamin A, vitamin B_6 and vitamin E, no statistically significant differences were observed between the corn event MZHG0JG hybrid (unsprayed) and the unmodified control corn hybrid for vitamins. In addition to this, except for vitamin A, vitamin B_1 and vitamin E, no statistically significant differences were observed between the corn event MZHG0JG (sprayed) and the unmodified control corn hybrid. However, these differences were not biologically significant as the means were within the range of the values observed in the reference corn varieties grown in the trials and the published literature (ILSI, 2014). No statistically significant differences were observed between the grain from corn event MZHG0JG hybrid and the unmodified control corn hybrid (both sprayed and unsprayed) for amino acids except for aspartic acid, arginine and tryptophan. Additionally a statistically significant difference was found for lysine in corn event MZHG0JG hybrid (sprayed) and the unmodified control corn hybrid. However, the means were within the range of the values observed in the reference corn grown in the trials and the published literature (ILSI, 2014). No statistically significant differences were observed between the grain samples from corn event MZHG0JG hybrid (unsprayed) and the unmodified control corn hybrid in the fatty acid except for heptadecanoic and linolenic fatty acids. Additionally, a statistically significant difference was found for palmitic acid in the corn event MZHG0JG hybrid (sprayed) and the unmodified control corn hybrid. However, all the means were within the range of the values observed between the reference corn grown in the trials and the published literature (ILSI, 2014). Except for p-coumaric, no statistically significant differences were observed between the corn event MZHG0JG hybrid (unsprayed) and unmodified control corn hybrid for secondary metabolites. Additionally, statistically significant differences were observed for inositol and p-coumaric between the corn event MZHG0JG hybrid (sprayed) and the unmodified control corn hybrid. However, all the means were within the range of values observed between the reference corn grown in the trials and the published scientific literature (ILSI, 2014). No statistically significant differences were observed between the grain from corn event MZHG0JG hybrid and the unmodified control corn hybrid (both sprayed and unsprayed) for the anti-nutrients.

Conclusion

It was concluded, based on the evidence provided by Syngenta Canada Inc. that the nutritional composition of the corn event MZHG0JG hybrid is similar to that of the unmodified control corn hybrid grown in the same trials and to that reported for other corn in the published scientific literature. Feed ingredients derived from corn event MZHG0JG are considered to meet present ingredient definitions for corn in Schedule IV of the Feeds Regulations.

2. Potential Impact of Corn Event MZHG0JG 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 MZHG0JG is tolerant to glyphosate due to production of the mEPSPS protein, and tolerant to glufosinate-ammonium due to production of the PAT (pat) protein:

The assessment of corn event MZHG0JG 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 mEPSPS protein
- The novel PAT protein
- The chemical pesticide residue profile

mEPSPS Protein

To obtain sufficient quantities of mEPSPS protein for assessment of environmental and feed safety, it was necessary to express the mepsps gene in a microbial production system. Equivalency was demonstrated between corn event MZHG0JG-produced mEPSPS protein and an E. coli-produced mEPSPS protein by comparing their molecular weights, immunoreactivity, glycosylation, N- and C-terminal sequence analysis, liquid chromatography-tandem mass spectrometry, and functional activity. Based on the results, the two proteins were found to be equivalent. Demonstration of equivalence between the mEPSPS protein produced in E. coli and the mEPSPS protein produced in corn event MZHG0JG allows the mEPSPS protein produced in E. coli to be used in studies to confirm the safety of the mEPSPS protein produced in corn event MZHG0JG.

The potential allergenicity and toxicity of the mEPSPS 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 mepsps gene, Z. mays, is not known to produce allergens and a bioinformatics evaluation of the mEPSPS protein amino acid sequence confirmed the lack of relevant similarities between this protein and known allergens. Unlike many allergens, studies with an E. coli-produced mEPSPS protein indicated that this protein is rapidly degraded in simulated gastric and intestinal fluid and not heat stable, and studies with corn event MZHG0JG-produced mEPSPS protein indicated that it is not glycosylated. The weight of evidence thus indicates that the mEPSPS protein is unlikely to be allergenic.

In terms of the potential toxicity to livestock, the mEPSPS 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 mEPSPS protein and known toxins. The amino acid sequence of the mEPSPS protein produced in corn event MZHG0JG was stated in the submission to be identical to a previously assessed mEPSPS protein. The livestock exposure to the mEPSPS protein is expected to be negligible as this protein is expressed at low levels in corn event MZHG0JG, 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 mEPSPS protein is unlikely to be toxic to livestock.

PAT Protein

To obtain sufficient quantities of PAT protein for assessment of environmental and feed safety, it was necessary to express the pat gene in a microbial production system. Equivalency was demonstrated between corn event MZHG0JG-produced PAT protein and an E. coli-produced PAT protein by comparing their molecular weights, immunoreactivity, glycosylation, liquid chromatography tandem mass spectrometry, and functional activity. Based on the results, the two proteins were found to be equivalent. Demonstration of equivalence between the PAT protein produced in E. coli and the PAT protein produced in corn event MZHG0JG allows the PAT protein produced in E. coli to be used in studies to confirm the safety of the PAT protein produced in corn event MZHG0JG.

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 known to produce allergens and a bioinformatics evaluation of the PAT protein amino acid sequences confirmed the lack of relevant similarities between this protein and known allergens. Unlike many allergens, studies with an E. coli-produced PAT protein indicated that this protein is rapidly degraded in simulated gastric fluid and intestinal fluid, and studies with corn event MZHG0JG-produced PAT protein indicated that it is not 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 sequences 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 MZHG0JG 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 MZHG0JG, is rapidly degraded under conditions which simulate the mammalian digestive tract and 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 MZHG0JG, following application of herbicides, were also evaluated as part of the feed safety assessment. It was determined that potential glyphosate or glufosinate-ammonium residues and their metabolites in livestock commodities derived from corn event MZHG0JG and its products would not present levels of concern to livestock, nor 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 novel mEPSPS and PAT protein-based herbicide tolerance traits will not confer to corn event MZHG0JG any characteristic that would raise concerns regarding the safety of corn event MZHG0JG. Feed ingredient(s) from corn event MZHG0JG 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 MZHG0JG 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 MZHG0JG 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 MZHG0JG.

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 MZHG0JG 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 mEPSPS and PAT protein-based herbicide tolerance traits will not confer to corn event MZHG0JG any characteristic that would raise any concerns regarding the safety or nutrition of corn event MZHG0JG. Grain corn, its by-products and corn oil are currently listed in IV of the Feeds Regulations and are, therefore authorized for use in livestock feeds in Canada. Corn event MZHG0JG has been found to be as safe as and as nutritious as currently and historically grown corn varieties. Corn event MZHG0JG 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 MZHG0JG 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 May 16th, 2016. Any corn lines derived from the corn event MZHG0JG 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.

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

Corn event MZHG0JG is subject to the same phytosanitary import requirements as unmodified corn varieties. Corn event MZHG0JG 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 MZHG0JG.

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