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## NOVEL FOOD INFORMATION

### AgriSureRW™ Insect-Protected Corn Event MIR604

Health Canada has notified Syngenta Seeds Inc. that it has no objection to the sale of food derived from corn lines containing the transformation event MIR604, which provides protection against feeding damage caused by the larvae of corn rootworm. The Department conducted a comprehensive assessment of this corn event according to its *Guidelines for the Safety Assessment of Novel Foods*. These Guidelines are based upon internationally accepted principles for establishing the safety of foods with novel traits.

### BACKGROUND:

The following provides a summary of the notification from AGBIOS, on behalf of Syngenta Seeds Inc., and the evaluation by Health Canada and contains no confidential business information.

#### 1. Introduction

Syngenta Seeds Inc. developed event MIR604 using recombinant DNA techniques to introduce two novel genes: *mcry3A*, a modified synthetic variant of the native *cry3A* gene originally derived from the common soil bacterium *Bacillus thuringiensis* var. *tenebrionis*, and the *pmi* gene encoding the phosphomannose isomerase (*pmi*) enzyme from the bacterium *Escherichia coli*, commonly found in the mammalian gut. The expression of the mCry3A protein provides protection from foraging damage by the larvae of corn rootworm species (*Diabrotica* sp.). The expression of the *pmi* enzyme allows the positive selection of transgenic corn cells from tissue culture media containing the sugar mannose.

The native *Cry3A* protein from *Bacillus thuringiensis* var. *tenebrionis* offers protection against the Colorado Potato Beetle (*Leptinotarsa decemlineata*), but exhibits little or no activity against other Coleopteran insect species. In contrast, the modified mCry3A protein contains an introduced cathepsin-G protease digestion site, which results in proteolytic activation of the insecticidal protein in the gut of corn rootworm species. Currently, other commercially grown corn exhibiting protection from corn rootworm are derived by expression of the *Cry3Bb1* protein from *Bacillus thuringiensis* subsp. *kumamotoensis*, or the *Cry34/35Ab1* proteins from *Bacillus thuringiensis* strain PS149B1.

The *pmi* enzyme is a new type of positive selectable marker that was developed as an alternative to antibiotic resistance or herbicide resistance marker genes used in previous submissions. *pmi* converts

mannose-6-phosphate to fructose-6-phosphate, thus allowing the use of the sugar mannose as a selective carbon source. *pmi* can only be used as a selectable marker for plant species lacking endogenous expression of the enzyme, such as corn. *pmi* enzymes are naturally found in mammals, insects, nematodes, bacteria, fungi, and many plant species with a history of safe use as human food such as pine, walnut, soybeans and other legumes.

The safety assessment performed by Food Directorate evaluators was conducted according to Health Canada's *Guidelines for the Safety Assessment of Novel Foods*. The assessment considered: how corn event MIR604 was developed; how the composition and nutritional quality of corn grain derived from plants containing this event compare to non-modified corn; and what the potential is for food products derived from plants containing this event to be toxic or cause allergic reactions.

The Food Directorate has a legislated responsibility for pre-market assessment of novel foods and novel food ingredients as detailed in Division 28 of Part B of the *Food and Drug Regulations (Novel Foods)*. Foods derived from corn lines containing event MIR604 are considered novel foods under the following part of the definition of novel foods: "c) a food that is derived from a plant, animal or microorganism that has been genetically modified such that

- i) the plant, animal or microorganism exhibits characteristics that were not previously observed in that plant, animal or microorganism".

## 2. Development of the Modified Plant

Corn event MIR604 was developed through *Agrobacterium* mediated transformation of corn embryo derived tissue followed by positive selection on culture media containing the sugar mannose as the sole or primary carbon source. The transforming plasmid pZM26 carried a transfer DNA (T-DNA) sequence comprised of two tandem expression cassettes, one for the mCry3A gene and one for the *pmi* gene. The mCry3A gene cassette contained a corn metallothionein-like (MTL) promoter, the coding region of synthetic mCry3A gene modified from the native *Cry3A* gene from *Bacillus thuringiensis* var. *tenebrionis*, and the 3' non-translated region of the nopaline synthase gene (NOS 3') from *Agrobacterium tumefaciens*. The *pmi* gene cassette contained the corn polyubiquitin promoter, including the first intron sequences (ZmUbi1nt), the coding region of the *pmi* gene from *Escherichia coli*, and the 3' non-translated region of the nopaline synthase gene (NOS 3') from *Agrobacterium tumefaciens*.

## 3. Characterization of the Modified Plant

Southern blot analysis of MIR604 demonstrated the insertion of a single copy of the tandem mCry3A and *pmi* expression cassettes in the maize genome at a single locus. Southern blot analysis also demonstrated the integrity of the genetic elements, as well as the absence of any plasmid backbone sequences outside the T-DNA region. The elements contained in the mCry3A and *pmi* expression cassettes have been shown to be stable with no rearrangements through Southern blot, PCR, and sequence analysis. Sequence analysis has also shown that both expression cassettes were entirely integrated into the corn genome and that all the elements are intact.

The stable inheritance of the single insert for event MIR604 is demonstrated by segregation analysis of progeny arising from selfing of the T4 generation. The presence of the mCry3A protein was detected by ELISA, while the

presence of the *mCry3A* and *pmi* genes was confirmed by PCR. Mendelian segregation ratios of 3:1 were obtained as expected for a single copy hemizygous trait for all above tests. Additionally, Southern blot analysis from three backcross generations demonstrated stability at the genotypic level for *mCry3A*.

#### 4. Product Information

Corn event MIR604 differs from its traditional counterparts by the addition of the *mCry3A* gene modified from *Bacillus thuringiensis* var. *tenebrionis* into the corn genome. The recombinant *mCry3A* protein is expressed in all corn tissues tested, including the root, the target tissue of foraging damage by corn rootworms. Corn event MIR604 also differs from its traditional counterparts by the addition of the *pmi* gene from *Escherichia coli*. The PMI enzyme serves as a selectable marker for post transformation selection on mannose media and does not supply any agronomic function in field grown plants.

#### 5. Dietary Exposure

MIR604 grain at seed maturity and senescence stages were sampled from one inbred and two hybrid lines in confined field trials. On a dry weight basis, the levels of *mCry3A* range from 0.8-2.0 µg/g, while the levels of PMI protein range from <0.07-0.5 µg/g.

#### 6. Nutrition

Test and control plants were grown over two growing seasons (2002 & 2003) at several locations within the USA and 1 location in Puerto Rico. Test and control plants were analyzed for 64 nutrients and anti-nutrients, in grain, as follows: **Proximates:** moisture, crude protein, crude fat, total fibre, ash, starch, carbohydrates; plus acid detergent fibre (ADF), neutral detergent fibre (NDF); **Minerals:** calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, zinc, selenium, chromium; **Fatty Acids:** palmitic, stearic, oleic, linoleic, linolenic acid; **Amino Acids:** asparagine, threonine, serine, glutamic acid, proline, glycine, alanine, cysteine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, histidine, lysine, arginine, tryptophan; **Vitamins:** β-carotene, cryptoxanthin, folic acid, B1 (thiamin), B2 (riboflavin), B3 (niacin), B5 (pantothenate), B6 (pyridoxine), E, α-tocopherol; **Phytosterols:** cholesterol, campesterol, stigmasterol, β-sisterol; **Anti-nutrients:** ferulic acid, p-coumaric acid, furfural, inositol, phytic acid, raffinose, trypsin inhibitor. 18 nutrients, in forage, were also analyzed.

For combined locations, 2002 and 2003, statistical differences were noted in 36 nutrients and 7 anti-nutrients in corn grain and forage. Differences occurring more than once in the same direction for the same nutrient indicating a trend (higher or lower) included: total protein (higher), vitamin B1 (higher), aspartic acid (higher), threonine (higher), serine (higher), glutamic acid (higher), alanine (higher), valine (higher), isoleucine (higher), leucine (higher), phenylalanine (higher), campesterol (higher), stigmasterol (higher), α-tocopherol (lower), ferulic acid (lower), p-coumaric acid (lower), zinc (lower) in grain and potassium (higher) in forage. However, all differences were within literature ranges. The nutrient composition of MIR604 is similar to conventional, commercial corn.

#### 7. Toxicology

The potential for toxicity for the novel proteins *mCry3A* and PMI expressed in

corn lines containing event MIR604 was considered remote. This conclusion was based on the low amounts of the novel proteins found in corn grain, the absence of demonstrated acute toxicity to the novel proteins in mice at doses orders of magnitude greater than the range associated with proteins, the lack of sequence homology between known toxins and the novel proteins, and the likelihood that the novel proteins will be degraded under conditions similar to those in the human gastrointestinal tract. There were no additional health concerns regarding endogenous toxins from food products derived from corn lines containing event MIR604 when compared to non-transgenic varieties.

The possibility that the novel proteins mCry3A and PMI would be allergenic in corn lines containing event MIR604 was also considered unlikely. This was based on the lack of sequence homology of mCry3A to any known allergens, the rapid digestion of the protein in simulated gastric fluids, and the loss of activity of the protein upon heat treatment. Some limited sequence homology was found between the PMI protein and two allergens, Hev b 13, from the latex of the rubber tree (*Hevea brasiliensis*) and  $\alpha$ -parvalbumin from an unidentified Indonesian frog of the genus *Rana*. However, studies with the serum of latex-sensitive patients have shown the allergenicity of rubber latex to be dependent on its glycosylated epitopes. PMI does not contain consensus sequences for N-glycosylation and is highly unlikely to be glycosylated. Additionally, tests with the serum of the single person known to have had an anaphylactic reaction to the frog allergen showed that the patient's IgE does not react with PMI, or with  $\alpha$ - or  $\beta$ -parvalbumin from a related species, *Rana esculenta*. Studies have also demonstrated that the PMI protein is rapidly digested in simulated gastric and intestinal fluid. Consequently, the available evidence suggests that PMI is highly unlikely to be an allergen.

These results suggest that foods derived from corn lines containing event MIR604 would not pose any greater allergenic risk than non-transgenic corn. At the expected level of consumption, there was no greater concern with corn lines containing event MIR604 than non-transgenic corn, with respect to its potential for toxicity or allergenicity.

### CONCLUSION:

Health Canada's review of the information presented in support of the food use of corn lines containing event MIR604 concluded that the food use of corn lines containing this event does not raise concerns related to safety. Health Canada is of the opinion that MIR604 is similar to non-transgenic parental strains of corn in terms of being an acceptable food source.

Health Canada's opinion deals only with the human food use of corn lines containing event MIR604. Issues related to growing corn lines containing event MIR604 in Canada and its use as livestock feed have been addressed separately through existing regulatory processes in the Canadian Food Inspection Agency.

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This Novel Food Information document has been prepared to summarize the opinion regarding the subject product provided by the Food Directorate, Health Products and Food Branch, Health Canada. This opinion is based upon the comprehensive review of information submitted by the petitioner according to the *Guidelines for the Safety Assessment of Novel Foods*.

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