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NOVEL FOOD INFORMATION - FOOD BIOTECHNOLOGY

POLLINATION CONTROL SYSTEM FOR CANOLA, MS1/RF2

Health Canada has notified Plant Genetic Systems N.V. that it has no objection to the food use of the transgenic canola lines MS1, RF2 or hybrids derived therefrom (PGS2), which have been developed to be tolerant to phosphinothricin containing herbicides, specifically glufosinate ammonium. The Department conducted a comprehensive assessment of MS1 and RF2 according to its *Guidelines for the Safety Assessment of Novel Foods* (September 1994). These guidelines are based upon internationally accepted principles for establishing the safety of foods derived from genetically modified organisms.

BACKGROUND:

The following provides a summary regarding the Plant Genetic Systems N.V. notification to Health Canada and contains no confidential business information.

1. Introduction

The MS1 and RF2 lines of canola (*Brassica napus*) were developed through a specific genetic modification of cultivar Drakkar to display the traits of nuclear male sterility and fertility restoration, respectively, and to be tolerant to glufosinate ammonium herbicide. The induction of male sterility (MS) was accomplished by insertion of the gene encoding barnase ribonuclease (RNase), and enzyme that disrupts RNA production and thus normal cell functioning resulting in arrested anther development. The trait of restored fertility (RF) was due to the introduction of the gene encoding a specific inhibitor of the

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For further information, please contact:

Office of Food Biotechnology
Food Directorate
Health Protection Branch
Health Canada
Tunney's Pasture
Ottawa, Ontario K1A 0L2

Telephone: (613) 941-5535
Facsimile: (613) 952-6400

barnase RNase. Only hybrids derived from the crossing of MS1 and RF2 are fertile. Both of the novel varieties, MS1 and RF2, contain a gene encoding the enzyme, phosphinothricin N-acetyl transferase (PAT), from *Streptomyces hygroscopicus*. Phosphinothricin containing herbicides, such as glufosinate ammonium, act by inhibiting glutamine synthetase resulting in the accumulation of toxic levels of ammonia. The PAT enzyme detoxifies phosphinothricin by acetylation into an inactive compound. Hybrids derived from MS1 and RF2 crosses permit farmers to use phosphinothricin containing herbicides for weed control in the cultivation of canola.

2. Development of the Modified Plant

The MS1 and RF2 canola lines were created by *Agrobacterium*-mediated transformation in which the transfer-DNA (T-DNA) contained either the *barnase* or *barstar* genes from the common soil bacterium, *Bacillus amyloliquefaciens*, under the control of the PTA29 anther-specific promoter from *Nicotiana tabacum*. In addition, each T-DNA contained a copy of the *bar* gene from *S. hygroscopicus*, which encodes the PAT enzyme, and sequences encoding the enzyme neomycin phosphotransferase II (NPTII) from the Tn5 transposon of *Escherichia coli*, strain K12. Expression of the *bar* gene was regulated by the PSsuAra promoter from *Arabidopsis thaliana* and post-translational targeting of the gene product to the chloroplast organelles was accomplished by fusion of the 5'-terminal coding sequence with the chloroplast transit peptide DNA sequence from *A. thaliana*. The expression of NPTII activity, under control of the nopaline synthase promoter from *A. tumefaciens*, was used as a selectable trait for screening transformed plants for the presence of the *barnase* and *barstar* genes, respectively. There was no incorporation of translatable plasmid DNA sequences outside of the T-DNA region as verified by Southern blot analysis. Data from several generations of backcrossing have demonstrated stable inheritance of the novel traits.

3. Product Information

Transcription of the *bar* gene was detected in both leaf and flower bud tissue by Northern blot analysis and was estimated to be 0.8-1.6 pg/µg total RNA and 0.1-0.2 pg/µg total RNA, respectively. Similar analyses performed on canola line RF2 demonstrated that the *barstar* gene was only transcribed in flower bud tissue at a level of 0.4-0.8 pg/µg total RNA. These novel varieties meet the standards for canola oil in Canada of containing less than 2% erucic acid and less than 30 µmoles/g glucosinolates in the oil-free meal. Other than the traits of glufosinate herbicide tolerance and either male sterility or fertility restoration, respectively, the disease, pest and other agronomic characteristics of MS1 and RF2 canola were comparable to non-transgenic Drakkar canola.

4. Dietary Exposure

The human consumption of canola products is limited to the refined oil. Refined edible canola oil does not contain any detectable protein and consists of purified triglycerides (96-97%). As the introduced gene products are not detectable in the refined oil produced from transgenic canola, there will be no human exposure to these proteins based on normal consumption patterns.

5. Nutrition

The analysis of nutrients from transgenic MS1 and RF2 canola and non-transgenic canola did not reveal any significant differences in the levels crude protein, crude fat, crude fibre, ash and gross energy in either whole seed or processed meal. The fatty acid composition of oils extracted from both transgenic and non-transgenic canola was statistically identical and within the normal range for canola oil. The consumption of refined oil from MS1, RF2 or hybrids derived therefrom will have no significant impact on the nutritional quality of the Canadian food supply.

6. Safety

Since only the processed oil from transgenic MS1, RF2, or lines derived therefrom, will be available for human consumption and the processing removes proteinaceous material, there are no additional toxicity or allergenicity concerns with this product.

CONCLUSION:

Health Canada's review of the information presented in support of the food use of glufosinate herbicide tolerant canola lines MS1 and RF2 concluded that they do not raise concerns related to human food safety. Health Canada is of the opinion that processed oil from MS1, RF2, or hybrids derived therefrom, is as safe and nutritious as that available from current commercial canola varieties.

Health Canada's opinion pertains only to the food use of these glufosinate herbicide tolerant canola lines. Issues related to growing glufosinate herbicide tolerant canola lines, with either male sterility or fertility restoration traits, in Canada and their use as animal feed are addressed separately through existing regulatory processes in the Canadian Food Inspection Agency.

This Novel Food Information document has been prepared to summarize the opinion regarding the subject product provided by the Food Directorate, Health Protection 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*.

(Également disponible en français)

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