Health Canada Santé Canada

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NOVEL FOOD INFORMATION - FOOD BIOTECHNOLOGY

INSECT RESISTANT CORN, 176

Health Canada has notified CIBA-Geigy Canada Ltd. (now Novartis Inc.) that it has no objection to the food use of the transgenic corn line 176, which has been developed to be resistant to insects, particularly the European Corn Borer (ECB). The Department conducted a comprehensive assessment of line 176 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 CIBA-Geigy Canada Ltd. notification to Health Canada and contains no confidential business information.

1. Introduction

The 176 line of corn (*Zea mays*) was developed through a specific genetic modification to be ECB (*Ostrinia nubilalis*) resistant. The novel variety produces a truncated version of the insecticidal protein, CryIA(b) derived from *Bacillus thuringiensis*. Delta-endotoxins, such as the CryIA(b) protein expressed in 176 corn, act by selectively binding to specific sites localized on the brush border midgut epithelium of susceptible insect species. Following binding, cation-specific pores are formed that disrupt midgut ion flow and thereby cause paralysis and death. CryIA(b) and related endotoxins are insecticidal only to lepidopteran insects and their specificity of action is directly attributable to the presence of specific binding sites in the target insects. There are no binding sites for delta-endotoxins of *B. thuringiensis* on the surface of mammalian intestinal cells, therefore, livestock animals and humans are not susceptible to these proteins. The modified corn line is protected from ECB damage which is a major insect pest in corn agriculture.

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2. Development of the Modified Plant

The 176 corn line was created through direct DNA transformation of immature embryos from the inbred line, CG00526, by microprojectile bombardment with DNA coated gold particles and regeneration of plants by tissue culture on selective medium. Two plasmids were used for transformation, one of which contained two copies of a 3' truncated *cryIA(b)* gene, each regulated by different promoter sequences. The *cryIA(b)* open reading frame, corresponding to the sequence encoding the N-terminal 648 amino acids of the native CryIA(b) protein, was modified for optimal expression in plant cells. Green tissue expression of one copy of the *cryIA(b)* gene was regulated by the phophoenolpyruvate carboxylase promoter while expression of the other *cryIA(b)* gene was controlled by a pollen specific promoter isolated from corn. Both genes employed 3'-polyadenylation sequences from the 35S transcript of cauliflower mosaic virus (CaMV). This plasmid also contained a copy of the β-lactamase encoding *bla* gene under control of a bacterial promoter. The *bla* gene was not expressed in plant cells, but was employed as a selectable trait for screening bacterial colonies for the presence of the plasmid vector. The second plasmid contained a copy of the *bar* gene from the soil bacterium *Streptomyces hygroscopicus* which encodes the phosphinothricin N-acetyl transferase (PAT) enzyme. This enzyme is used as a selectable marker and confers resistance to glufosinate ammonium herbicide. Constitutive expression of the *bar* gene was under the control of the CaMV 35S promoter. Apart from the sequences encoding Cry1A(b) and PAT, no other plant translatable DNA sequences were introduced into the plant genome. Data from several generations of backcrossing demonstrated that the *cryIA(b)* and *bar* genes were tightly linked and stably inherited.

3. Product Information

The expression level of the CryIA(b) protein was determined in the leaves, pollen, roots and kernels of transgenic corn. The highest concentrations of CryIA(b) were observed in the leaves of plants in the vegetative or anthesis stages (0.53 – 3.03 μg/g fresh weight tissue) and declined with increasing maturity and plant senescence. The CryIA(b) protein was present at concentrations of 1.14 and 2.35 μg/g fresh weight tissue, and was detectable at trace levels (<0.008 μg/g fresh weight) in roots and kernels. While Southern blot analysis confirmed the presence of the *bar* gene in all plant tissues, expressed PAT protein was undetectable in leaves, pollen, roots or kernels of transgenic corn at a detection threshold of 0.2 ppm. The hydroxamic acid, 2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-2(4H)-one (DIMBOA) is the only putative endogenous toxin from corn and is postulated to play a protective role against specific fungal and bacterial pathogens as well as insect pests. The level of DIMBOA is normally highest in the leaf tissue of young plants and is absent in kernels. The measured concentrations of DIMBOA in leaf tissue from transgenic 176 corn and untransformed control plants grown under identical conditions were statistically identical. No significant differences were observed between hybrids derived using original elite lines and the selected 176 line for the agronomic traits of yield, moisture at harvest, root lodging rating, ear height, plant height, heat units to silking or pollen shed. Other than resistance to ECB and tolerance to glufosinate ammonium herbicide, the disease, pest and other agronomic characteristics of 176 corn were comparable to non-transgenic lines of corn.

4. Dietary Exposure

The genetic modification of 176 corn will not result in any change in the consumption pattern for this product. Consequently, the dietary exposure of Canadians to this product is anticipated to be the same as for other lines of commercially available corn.

5. Nutrition

The analysis of nutrients from transgenic 176 corn and non-transgenic corn did not reveal any significant differences in the levels protein, fat, fibre and starch. Similarly, the levels of micronutrients including calcium, phosphorus, potassium and magnesium were within the established ranges for corn. The consumption of products from 176 corn will have no significant impact on the nutritional quality of the Canadian food supply.

6. Safety

a) Potential Toxicity:

Direct toxicity studies conducted using CryIA(b) and PAT test material did not reveal any deleterious effects. The amino acid sequence of the truncated CryIA(b) protein expressed in 176 corn is closely related the sequence of the same proteins that are present in strains of *B. thuringiensis* that have been used for over 30 years as commercial organic microbial insecticides. An analysis of the amino acid sequences of the inserted CryIA(b) protein and the PAT enzyme did not show homologies with known mammalian protein toxins and they are not judged to have any potential for human toxicity.

b) Potential Allergenicity:

The truncated CryIA(b) protein and the PAT enzyme expressed in 176 corn do not possess characteristics typical of known protein allergens. There were no regions of homology when the sequences of these introduced proteins were compared to the amino acid sequences of known protein allergens. Unlike known protein allergens, both of these proteins are rapidly degraded by acid and/or enzymatic hydrolysis when exposed to simulated gastric fluids. The Cry1A(b) and PAT proteins are extremely unlikely to be allergenic.

CONCLUSION:

Health Canada's review of the information presented in support of the food use of insect resistant 176 corn concluded that this corn does not raise concerns related to human food safety. Health Canada is of the opinion that products from 176 corn are as safe and nutritious as those available from current commercial corn varieties.

Health Canada's opinion pertains only to the food use of this insect resistant corn. Issues related to growing insect resistant corn in Canada and its use as animal feed are addressed separately through existing regulatory processes in the Canadian Food Inspection Agency.