Food and Nutrition

ARCHIVED - Novel Food Information - Drought Tolerant Corn - MON 87460

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Health Canada has notified Monsanto Canada Inc. that it has no objection to the sale of food derived fromDrought tolerant corn MON 87460. 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 Monsanto Canada Inc. and the evaluation by Heath Canada and contains no confidential business information.

1. Introduction

Monsanto has developed Drought Tolerant Corn MON 87460 using recombinant DNA techniques to introduce the cold shock protein B (*cspB*) coding sequence derived from the common soil bacterium *Bacillus subtilis*. The sequence codes for the CSPB protein which has been shown to bind to a broad array of RNA, allowing them to adopt the correct conformation under stress conditions and improve cellular function in the plant. Additionally, Drought Tolerant Corn MON 87460 was also genetically-modified to introduce the neomycin phosphotransferase II (*nptII*) coding sequence derived from a non-virulent strain of *Escherichia coli*. The sequence codes for the NPTII enzyme which confers resistance to the antibiotic kanamycin and it was used as a selectable marker.

The safety assessment performed by Food Directorate evaluators was conducted according to Health Canada's Guidelines for the Safety Assessment of Novel Foods. These Guidelines are based on harmonization efforts with other regulatory authorities and reflect international guidance documents in this area (e.g., Codex Alimentarius). The assessment considered: how MON 87460 corn was developed; how the composition and nutritional quality of MON 87460 corn compared to non-modified varieties; and what the potential is for MON 87460 corn to be toxic or cause allergic reactions. Monsanto has provided data which demonstrates that MON 87460 corn is as safe and of the same nutritional quality as traditional corn varieties used as food in Canada.

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 MON 87460 corn 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

The petitioner has provided information describing the methods used to develop MON 87460 and molecular biology data that characterizes the genetic change which confers tolerance to drought. Drought tolerance was achieved by transformation of the conventional corn variety LH59 with a transgenic expression cassette containing the novel genes *cspb* (cold shock protein B) and *nptII* (neomycin phosphotransferase II) and their associated regulatory elements.

The CSPB protein is an RNA chaperone protein from *B. subtilis*, which is associated with enhanced stress acclimation and tolerance by unfolding misfolded RNA secondary structures, thereby facilitating RNA translation. Similar to bacteria, Cold Shock Domain (CSD)-containing proteins in plants also bind RNA, unfold RNA secondary structures caused by environmental stress, and help maintain cellular functions under stress. These plant CSD-containing proteins share a high level of similarity to the bacterial CSPs and have been shown to share *in vitro* and *in vivo* functions with bacterial CSPs. Proteins containing CSDs are present across bacteria, plants, and animals, implying that proteins with the ability to bind RNA transiently and non-specifically may-be necessary for nearly all forms of life. Plant CSD-containing proteins have been reported to respond to abiotic stresses in Arabidopsis, wheat, and rice, and to play an important role in various aspects of plant development.

Direct relationships between the ability of CSD-containing proteins to bind RNA and/or ssDNA and stress tolerance have been established and results of *in vitro* experiments show that plant CSD-containing proteins can bind RNA, synthetic mRNA, and ssDNA. It is also known that RNA chaperones such as CSPs and plant CSD-containing proteins bind without sequence specificity. The absence of binding sequence specificity indicates that plant CSD-containing proteins could be involved in a more general response to stress by binding RNAs and, therefore, help cells to maintain cellular functions following the stress. CSD-containing proteins from rice and *Arabidopsis* have been shown to be highly expressed in apical meristems, ovules, embryos, and seeds and, therefore, could potentially affect growth rate, flowering time, and seed development. The CSD-containing proteins have been localized both in the cytoplasm and the nuclei, indicating that these proteins can potentially be involved in multiple aspects of RNA function including localization, translation and stability.

MON 87460 corn was produced using *Agrobacterium*-mediated transformation of commercial corn variety LH59 with the transformation vector PV-ZMAP595. The transformation vector PV-ZMAP595 was used to introduce the *cspb* and *nptII* genes and their associated regulatory elements. This modification process is expected to result in the transfer of only those genetic elements contained between the left and right border regions in PV-ZMAP595. The following elements were transferred into the commercial corn variety LH59:

the promoter and leader from the rice actin gene, *act1* (*P-Ract1*), the intron from the rice actin gene, act1 (*I-Ract1*), the coding sequence of the *cspB* gene from *Bacillus subtilis* encoding CSPB (*CS-cspB*), the 3' nontranslated sequence of transcript 7 gene from *Agrobacterium tumefaciens* that directs polyadenylation (*T-tr7*), the sequence from Bacteriophage P1 for the recombination site recognized by Cre recombinase (*loxP*), the promoter for the 35S RNA of the Cauliflower Mosaic Virus (*P-35S*), the coding sequence from Tn5 in *E. coli* encoding neomycin and kanamycin resistance (*CS-nptII*), the 3' nontranslated sequence of the nopaline synthase (NOS) gene from *Agrobacterium tumefaciens* which terminates and directs polyadenylation (*T-nos*), and the sequence from Bacteriophage P1 for the recombination site recognized by Cre recombinase (*loxP*)

3. Characterization of the Modified Plant

Southern blot analysis of MON 87460 demonstrated the insertion of a single copy of the genetic elements contained between the left and right border regions in PV-ZMAP595 in the corn genome at

a single locus. The insertion fragment is composed of intact copies of the *cspB* and *nptII* gene cassettes. Southern blot analysis confirmed the absence of backbone DNA in MON 87460 corn.

The petitioner has provided both southern blots and segregation data to demonstrate the inserted T-DNA is stable and inherited in the expected manner. Data were presented for six generations of MON 87460 corn. The data presented indicate that, at the genetic level, the introduced elements are stably integrated. The segregation data presented by the petitioner demonstrated that the trait is stable phenotypically and is inherited in the expected Mendelian manner.

4. Product Information

Corn event MON 87460 differs from conventional corn by the insertion of two novel genes; *cspb* and *nptII* and their associated regulatory elements. The insertion of these genes results in the expression of two novel proteins in MON87460; CSPB and NPTII. The expression of CSPB confers resistance to drought stress conditions.The expression of NPTII in MON87460, confers resistance to the antibiotic kanamycin and was used a selectable marker for transformed plants.

The petitioner has provided data to demonstrate the level of expression of the CPSB in MON87460. This study used plant samples from six field sites planted in the 2006 growing season in the major corn growing locations in the United States. Each site was planted in three replicated plots using an complete block design. The quantities of CSPB protein were determined by an enzyme-linked immunosorbent assay (ELISA). Protein quantities for the tissues were calculated on a microgram (µg) per gram (g) fresh weight (fwt) basis. Moisture content was measured for all tissue types, and protein quantities from these tissues were converted to dry weight (dwt) values. Both sets of values were presented by the petitioner. The range of CSPB expressed in grain from US-grown MON 87460 was 0.040 - 0.089 µg/g fwt, with a mean value of 0.063 µg/g fwt.

In addition to the field studies collected in the United States, the petitioner has also provided the results of a single field trial from the 2006/2007 growing season in Chile. This trial was conducted to determine if differences in water conditions altered the protein expression levels. Data were collected for expression in all the previously assessed tissues under well-watered and water-limited conditions. Results from this trial were presented on a fresh-weight (fwt) and dry-weight basis (dwt). Protein expression levels, both the mean and range values, were shown to be comparable between the US and Chilean trial sites and were shown to be comparable between the well-watered and water-limited conditions.

The petitioner has provided data to demonstrate the level of expression of the NPTII in the altered corn. This study used plant samples from six field sites planted in the 2006 growing season in the major corn growing locations in the United States. Each site was planted in three replicated plots using a complete block design. The quantities of CSPB protein were determined by an enzyme-linked immunosorbent assay (ELISA). Protein quantities for the tissues were calculated on a microgram (µg) per gram (g) fresh weight (fwt) basis. Moisture content was measured for all tissue types, and protein quantities from these tissues were converted to dry weight (dwt) values. Both sets of values were presented by the petitioner. For grain, the levels of NPTII expressed in MON87460 were below the limit of quantification (0.0047 µg/g fwt) and therefore no range or mean values were presented.

In addition to the field studies collected in the United States, the petitioner has also provided the results of a single field trial from the 2006/2007 growing season in Chile. This trial was conducted to determine if differences in water conditions altered the protein expression levels. Data was collected for expression in all the previously assessed tissues under well-watered and water-limited conditions. Results from this trial were presented on a fresh-weight (fwt) and dry-weight basis (dwt). Protein expression levels, both the mean and range values, were shown to be comparable between the US and Chilean trail sites and were shown to be comparable between the well-watered and water limited conditions. Levels of NPTII expression in the grain remained below the LOQ under both water conditions.

5. Dietary Exposure

Drought Tolerant Corn MON 87460 is expected to be used in similar application as traditional corn varieties by the food industry. The petitioner has indicated that the greatest use of corn grain in food is the production of starch and sweetener products through wet milling. Dry milling is also used to produce corn grits, flour and meal, although the greatest use of dry milled corn is in brewing beer.

6. Nutrition

The petitioner provided data from corn grown at six sites: two in Iowa, and one in each of Illinois, Indiana, Kansas and Nebraska. Of these sites, four sites were rain-fed (IA, IA, IL, IN) and two received supplemental irrigation (KS, NE). In these trials, MON 87460 corn was compared to the parental control variety and 18 commercial corn varieties, under common corn production conditions. The data were obtained using an appropriate study design and accepted analytical methods.

Grain samples were analyzed for key components, including proximates (protein, fat, ash, moisture, carbohydrates (by calculation), acid detergent fibre, neutral detergent fibre, and total detergent fibre), total amino acid composition, fatty acid composition (C8-C22), minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium and, zinc) vitamins (vitamin B1(thiamine), vitamin B2 (riboflavin), vitamin B6, vitamin E, niacin and folic acid), anti-nutrients (furfural, raffinose, and phytic acid) and secondary metabolites (*p*-coumaric and ferulic acid).

Statistically significant differences were identified in three components analysed: ash, stearic acid, and eicosanoic acid. For stearic acid, no significant differences were noted between MON 87460 and the control variety at individual sites. Ash and eicosanoic acid were significantly different at two sites; however, their values were within the International Life Sciences Institute Crop Composition Database conventional corn ranges and were similar to values at other field sites.

The petitioner also provided the result of studies, repeating the US studies, conducted in Chile at four field trial sites, under well-watered and water-limited conditions. Under well-watered conditions, when data from all sites were combined, two components, magnesium and fat, were significantly higher in MON87460 than the control. Individual site comparisons for both components showed significant differences at only one site. No other differences were observed. Under water-limited conditions, when data from all sites were combined, the only significant difference was that levels of eicosanoic acid were lower in MON87460 than the control. The individual site comparisons showed a significant difference in eicosanoic acid at a single site. No other differences were observed.

Corn from Chile field sites was also analysed for the following metabolites: osmoprotectants (sugar and polyols (sucrose, glucose, fructose, sorbitol, mannitol, and glycerol), free proline, glycine betaine, and choline and metabolites generally associated with stress responses (salicylic acid and abscisic acid). There were no significant differences in individual or combined site analysis for grain samples of MON 87460 vs. control under well-watered conditions. Under water-limited conditions, in the combined site analysis, only one metabolite (sucrose) was reported to be different (lower) in MON87460 vs. control. This difference was observed in two of the individual sites.

In summary, the compositional analysis from both US and Chilean field trials, demonstrated that there were very few statistically significant differences between MON 87640 and the control lines (fat, one fatty acid, one mineral, sucrose). The levels of these analytes were within their respective reference ranges. As well, the differences were not consistent across sites. The differences observed are likely due to natural variation and would have no impact on nutritional quality.

A 45-day broiler feeding study was conducted to examine the impact of feed derived from MON 87460 on performance and meat composition. The study used 100 birds per treatment (MON97460 vs. control, as well as various other corn varieties). Carcass yield measurements were not different

for broilers fed diets containing MON 87460 compared to those fed diets containing conventional control corn. Average carcass measurements were within the range observed for birds fed diets formulated to the same nutrient specifications using 6 conventional reference corn hybrids.

Meat analysis results for breast moisture, breast protein, breast fat, thigh moisture, thigh protein and thigh fat were not different for birds fed diets containing MON 87460, as compared to those of birds fed diets containing conventional control or reference corn based on individual diet comparisons or comparison to the population of conventional control and reference corn diets.

7. Toxicology

The novel gene *cspb,* is derived from the non-pathogenic microorganism *Bacillus subtilis*. *B. subtilis* is present in many fermented foods, and has a history of safe consumption. In Canada, *B. subtilis* is a permitted source organism for enzymes used in a variety of food products. .

In an acute toxicity study, no adverse effects were seen in mice administered CSPB protein at 4.7 mg/kg bw and this dose was considered to be the NOEL (No observed effect level). The 95th percentile consumers intake of the CSPB protein through corn and corn products was calculated as 176 and 413 ng/kg bw/day for the general population and children aged 1-6 years, respectively. Therefore, the margin of exposure between the NOEL in the study and estimated intake is about 26,700 and 11,400 for the general population and children aged 1-6 years, respectively.

Studies provided by the petitioner demonstrated that the protein is rapidly digested in simulated gastric (SGF) and intestinal (SIF) fluids, and that the protein is heat-labile. Based on this information, it is unlikely that CSPB expressed in MON 87460 would survive either the processing typical to corn or digestion.

The CSPB protein was evaluated for its potential for allergenicity using amino acid homology searches. The amino acid sequence of CSPB was compared to all the sequences in the Allergen database, version 8 (AD8) using the FASTA sequence alignment tool. The CSPB sequence was also evaluated against the sequences in the AD8 using pairwise comparison algorithm (ALLERGENSEARCH) to identify any sequences of 8 or more linearly contigous amino acids that could possibly be indicative of a shared and potentially cross-reactive epitope. FASTA identified no alignments between CSPB and any allergenic protein in the AD8 that would indicate significant similarity. The search algorithm ALLERGENSEARCH revealed no shared sequences of 8 amino acids.

The CSPB protein was used as the query protein in a standard FASTA search for sequence alignment similarity with all the known protein sequneces in GenBank's PROTEIN database. CSPB was similarly evaluated against all the sequences of the TOXIN6 database. The FASTA program identified 5,242 proteins in the PROTEIN database that showed some similarity to CSPB. These alignments were shown to be naturally occurring or theoretical proteins described as cold shock proteins, i.e., they were homologous sequences to CSPB. The FASTA search of the TOXIN6 database found no proteins that were similar to CSPB.

Health Canada also evaluated the possibility that CSPB might have sequence homology to Cla h8 CSP protein, a purported "minor allergen" of *Cladosporium herbarum*. This mold is a common source of air-borne allergy. When CSPB from *B. subtilis* was aligned with the Cla h8 CSP protein, with one amino acid exception, there was a 35-amino acid sequence homology between the proteins. When asked to comment on this issue, the petitioner provided a scientific argument, relying on the published literature, that Cla h8 CSP is not a known allergen, has not been reviewed or recognised by the Allergen Nomenclature Subcommittee of the International Union for Immunological Sciences as such, and is not homologous to any know allergen itself. They also restated the previous evidence that CSPB does not display any of the characteristics of known food allergens. Health Canada evaluators reviewed the evidence provided and concluded that there is no evidence that Cla h8 CSP is an allergen, and the likelihood that it is a food allergen is negligible.

The novel gene *nptII*, derived from a non-pathogenic strain of *E. coli*, is one of the most commonly

used antibiotic resistance markers in commercially grown genetically-modified crops, including several approved by Health Canada. The petitioner demonstrated the equivalence of the NPTII protein expressed in MON 87460 to protein used in previously submitted studies demonstrating the safety of this protein.

In a previously submitted acute toxicity study for NPTII, mice were administered the protein at 5000 mg/kg bw, which was determined to be the No Observed Adverse Effect Level (NOAEL). Previous studies have also shown NPTII to be rapidly degraded in SGF and SIF.

The petitioner submitted newly conducted amino acid homology searches to update and confirm the previously submitted studies on allergenicity. The petitioner submitted result for FASTA and ALLERGENSEARCH searches of the Allergen Database, version 8 (AD8), using NPTII as the query protein. FASTA revealed no similarities between the NPTII protein and any known allergen in the AD8 database. ALLERGENSEARCH revealed that NPTII shares no sequences of 8 contiguous amino acids with any allergen in the database.

Conclusion:

Health Canada's review of the information presented in support of the food use of Drought tolerant corn MON 87460 concluded that derived food products do not raise concerns related to safety. Health Canada is of the opinion that MON 87460 is similar to regular commodity corn in terms of being an acceptable food source.

Health Canada's opinion deals only with the human food use of Drought tolerant corn MON 87460. Issues related to the environmental safety of Drought Tolerant Corn MON 87460 in Canada and its use as livestock feed have been 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 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*.

(Également disponible en français)

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