
U. S. Food and Drug Administration
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
December 31, 2001

Biotechnology Consultation

Note to the File

BNF No. 000075

Date: December 31, 2001

Subject: Monsanto's Corn Rootworm Protected Corn, MON 863 transformation event.

Keywords:

Corn, *Zea mays*, corn rootworm, *Diabrotica sp.*, Cry3Bb1, *Bacillus thuringiensis* (subspecies *kumamotoensis*), Coleopteran-specific insecticidal protein, *nptII* gene, MON 863

Background

In a submission dated September 25, 2000, as amended on August 20, 2001, Monsanto provided FDA with its summary of the nutritional and safety assessment of a new insect protected corn line containing the transformation event MON 863. The firm initiated the consultation with the agency regarding this product on September 1, 2000.

Intended Effect of the Genetic Modification and Food/Feed Use

According to Monsanto, the intended technical effect of the genetic modification of the corn, *Zea mays*, is to protect corn plants from damage by corn rootworm (CRW) feeding. CRW larvae feed on the roots of corn plants reducing the ability of the plant to absorb nutrients and water from the soil, and cause harvesting difficulties due to plant lodging. CRW (Coleopteran, *Diabrotica sp.*) is a significant insect pest problem for corn production in the U.S. corn belt. To confer protection against CRW, Monsanto used a modified *cry3Bb1* gene derived from *Bacillus thuringiensis* subspecies *kumamotoensis* (*B.t.k.*), to express a *B.t.k.* Cry3Bb1 protein that is selectively toxic to Coleopteran species. The modified *cry3Bb1* gene that is expressed in the MON 863 corn line differs from the wild-type *cry3Bb1* gene by the addition of an alanine residue at position 2 of the protein, and by seven amino acid changes. There are 653 amino acids in the full length protein.

Corn grain and its processed fractions are consumed as human food and animal feed. Corn is a raw material in the manufacture of starch which is used as starch product or for the production of high fructose corn syrup and ethanol. Corn oil is processed from the germ. Each of these materials is a component of many foods including bakery and dairy goods, beverages, confections, and meat products. Approximately two-thirds of the corn produced in the U.S. is fed to livestock. Grain is fed directly to livestock. Wet and dry milling by-products (primarily corn gluten meal and feed) are also fed directly or used in feeds. Corn forage is extensively consumed as an animal feed by ruminants. The introduction of

the *cry3Bb1* gene and the *nptII* marker gene are not intended to alter the food and feed uses of corn.

Molecular Alterations and Characterization

Monsanto used a particle acceleration method to introduce a purified, linear DNA into the germplasm of the publicly available inbred line of corn, A634. Monsanto reported that line A634 was used because it responds well to particle bombardment transformation and tissue culture regeneration. As well, it is among the most popular public inbreds used in U.S. hybrid corn production.

The linear DNA vector, PV-ZMIR13L, was prepared by restriction endonuclease digestion (*Mlu* I) of the plasmid, PV-ZMIR13. The linear vector contained the modified *cry3Bb1* gene derived from *B.t.k.*, and the selectable marker gene, *nptII*, derived from the *Escherichia coli* transposon, Tn5. The expression cassette consists of the modified *cry3Bb1* coding region under the control of the 4-AS1 plant promoter (four repeats of an activating sequence and a single portion of the 35S promoter) derived from the cauliflower mosaic virus (CaMV), and the 5' untranslated leader sequence of wheat chlorophyll a/b binding protein (wt CAB leader), the rice actin intron, and the 3' transcriptional termination sequence derived from the 3' untranslated sequence of the gene encoding the wheat heat shock protein 17.3 (tahsp17). The *nptII* expression cassette consists of the *nptII* coding region regulated by the 35S promoter derived from CaMV and the untranslated 3' transcription termination sequence (NOS 3') from the *Agrobacterium tumefaciens* nopaline synthase gene. The DNA fragment containing the *nptII* gene from the bacterial transposon, Tn5, also contains a 153 base pair (bp) portion of the 378 bp bleomycin binding protein gene (*ble*).

Monsanto performed a molecular analysis using Southern blotting and polymerase chain reaction (PCR) to characterize the MON 863 insertion event. Monsanto stated that the results of these analyses demonstrate that a single copy of the linear DNA vector, PV-ZMIR13L, is integrated at a single site in the corn genome; the modified *cry3Bb1* gene, the *nptII* gene, and their associated promoters and terminators were intact; no additional DNA sequences derived from the plasmid, PV-ZMIR13, could be detected.

Monsanto examined the segregation and stability of the MON 863 event by analyzing segregation data for the CRW-protected phenotype over five generations; performing enzyme linked immunosorbant assay (ELISA) for the expression of Cry3Bb1 protein on plants identified as being positive for CRW-protected phenotype; and by doing Southern blot analysis of DNA extracted from plants spanning three generations. Monsanto concluded that the results of these tests demonstrated the stability of the inserted DNA in MON 863 across multiple generations.

Expressed Proteins

Two new proteins, a modified Cry3Bb1, and the NptII enzyme, are expressed in the transgenic corn line MON 863. Monsanto reported the results of analysis by ELISA, and as expected, the modified Cry3Bb1 protein is expressed in the tissue of young leaf, grain, mature root, forage, silk, and pollen. Mean levels of the modified Cry3Bb1 protein ranged from 10 to 81 micrograms/g fresh weight of plant tissue, depending on the tissue examined and time of harvest. Upon examination of the tissue of young leaf, forage, and grain by ELISA, NptII enzyme was detected in leaf and forage. Mean levels of NptII protein ranged from not detectable (<0.076 micrograms/g) to 1.4 micrograms/g.

Monsanto discussed how differences in the initiation of translation between prokaryotes and eukaryotes make it highly unlikely that the partial *ble* gene, located twenty nucleotides downstream of the stop codon for the *nptII* gene, would be translated into protein. Monsanto stated that if the partial *ble* gene

were translated into protein, the truncated peptide would not dimerize because it lacks the necessary amino acids to dimerize, and also lacks approximately 50% of the residues that are involved in bleomycin binding.

Regulatory Considerations

The safe use of pesticidal substances as well as the use of selectable markers as inert ingredients in the development of pest-resistant plant varieties is under the regulatory purview of the Environmental Protection Agency (EPA). Thus EPA regulates the use of the insecticidal protein, Cry3Bb1, and the selectable marker NptII, as well as the genetic material encoding them. Therefore, although Monsanto presented information regarding these proteins, including expression levels, we have not addressed the safety of the use of these proteins. The main focus of this consultation is on compositional analysis of this transgenic corn as compared to the parental or other commonly consumed varieties.

Compositional Analysis

Monsanto conducted compositional analyses on tissues collected from the MON 863 event, its nontransgenic parental control line at four replicated sites, and nine different nontransgenic, commercial corn hybrids grown under field conditions at two replicated sites each. Field trials were conducted at four different sites in the United States. Forage and grain samples were collected from all sites.

Grain samples were analyzed for proximate (protein, fat, ash, moisture), acid detergent fiber (ADF), neutral detergent fiber (NDF), amino acids, fatty acids, vitamin E, minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc), phytic acid, and trypsin inhibitor. Carbohydrate levels were determined by calculation. Levels of carbohydrates and the non-essential amino acids cystine, aspartic acid, and glycine were higher in the MON 863 than in the control variety, but were within the range of levels for the nine commercial varieties. However, levels of protein, the essential amino acids leucine and phenylalanine, and the non-essential amino acid glutamic acid were lower in MON 863 than in the control variety, yet they were well within the range of values for the nine control varieties. Further, the levels of the minerals phosphorus, magnesium, zinc and manganese, and vitamin E were also lower in MON 863 than in the control variety, but were within the range of values for the nine control varieties and those reported in the literature. The levels of all the other nutrients measured were not different from the control variety, and the levels were within the range of values for the nine control varieties and those reported in the literature. The level of the antinutrient, phytic acid, was also lower in MON 863 than in the control variety and within the range of values for the nine control varieties.

Forage samples were analyzed for proximate, ADF, and NDF. Carbohydrate levels were determined by calculation. There were no significant differences between levels of these nutrients present in MON 863 than in the control variety, and all values fell within the range reported for the nine commercial varieties and for the range of values reported in the literature.

Monsanto stated that these observations support a conclusion that the measured differences represent normal biological and analytical variability.

Conclusions

Monsanto has concluded that corn from transformed line MON 863 is not materially different in composition, safety or agronomic characteristics from nontransgenic lines of corn other than for its resistance to corn rootworm feeding damage. At this time, based on Monsanto's reporting of its data and

analyses, the Agency considers Monsanto's consultation on transgenic corn line MON 863 to be complete.

/s/
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Content last updated by mdd on 2002-MAR-01
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