DEPARTMENT OF HEALTH AND HUMAN SERVICES

NOTE TO THE FILE (BNF0034)
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Subject: Insect Protected Corn

Keywords:

Corn, Insect resistant, *Bacillus thuringiensis* subspecies *kurstaki*, *cryIA(b)*, Insect control protein CryIA(b), *Ostrinia nubilalis*, European Corn Borer, *hsp70* intron, Glyphosate tolerant, Herbicide tolerant, *CP4 EPSPS*, 5-enolpyruvylshikimate-3-phosphate synthase (CP4 EPSPS), *gox*, glyphosate oxidoreductase, *nptII*, neomycin phosphotransferase II (NPTII)

Background

In submissions dated June 6 and August 16, 1996, Monsanto Company provided summary information to support their safety and nutritional assessment of new corn lines (hybrids) containing transformation events MON809 and MON810.

Intended Effect and Food/Feed Use

The intended technical effect of this genetic modification of corn is to confer resistance to the feeding of the European Corn Borer, *Ostrinia nubilalis*, a lepidopteran pest insect. The modified corn lines also resist the feeding of another lepidopteran pest insect, the southwestern corn borer. Monsanto states that insect resistance is conferred by the insecticidal protein cryIA(b), which is reported to be identical to that found in nature and in commercial insecticidal formulations obtained from *Bacillus thuringiensis*. Tolerance to the herbicidal compound glyphosate was utilized as a selectable marker during the development of MON809 corn. The firm indicates that the protein conferring herbicide tolerance, 5-enolpyruvylshikimate-3-phosphate synthase (CP4 EPSPS), is equivalent to that present in modified soybean and cotton lines, about which Monsanto has previously informed the Agency. Monsanto states that MON809 corn will not tolerate application of commercially acceptable levels of glyphosate and thus, will be marketed only for its insect resistant qualities. Line MON810 does not contain the selectable marker which confers herbicide tolerance.

The firm reports that corn grain is used in many human foods, but 50 to 60% of the grain produced in the United States is used for animal feed. Human food products include starch, high fructose corn syrup, ethanol, and corn oil. Whole plant corn silage, which makes up 10-12% of annual corn acreage, is a major ruminant feedstuff.

Molecular Alterations and Characterization

The novel genetic material contained in lines MON809 and MON810 was inserted into the corn genotype, Hi-II, using particle acceleration transformation. The transformation vectors were plasmids PV-ZMBK07 and PV-ZMGT10.

Monsanto reports that there is only one insertion site in MON809 corn, as determined by Southern blot analyses and confirmed by Mendelian segregation data. The firm indicates that MON809 contains genetic material from both plasmids. The single insert contains 1 complete and 1 partial copy of the modified *cryIA(b)* gene. The line also contains a partial copy of the *gox* gene (which is unexpressed) and 2 complete copies of the *CP4-EPSPS* gene (expressed). In addition, the *ori-p*UC sequence and the *nptII* gene, present in both plasmids, have been incorporated into MON809 corn, but they were not of the predicted size. The gene *nptII* codes for neomycin phosphotransferase II (NPTII). NPTII is used as a selectable marker in bacterial systems. The firm states that *nptII* is under the control of a bacterial promoter and thus, will not be expressed in corn. The firm reports that the *cryIA(b)* gene was stable for 5 generations in MON809 corn.

Line MON810 was also analyzed using Southern blot techniques to determine the presence of novel genetic material. This line contains only one insert from plasmid PV-ZMBK07. The *CP4-EPSPS*, *gox*, and *nptII* genes are not present, nor is the *ori-p*UC sequence. MON810 contains 1 complete copy of the *cryIA(b)* gene and its associated regulatory sequences. Monsanto reports that Mendelian segregation analysis supports their conclusion of 1 active insert in this line. The insert is reported to be stable through 7 generations of crossing to a parental line. MON810 is considered to be an "escape" in that it grew under conditions that should have required the presence of herbicide tolerance for survival, but the line does not contain the necessary genetic material. Monsanto postulates that herbicide tolerant cells near MON810 degraded the herbicide, thus permitting MON810 survival.

The *cryIA(b)* gene is reported to be a modified version of the gene present in *Bacillus thuringiensis* subsp. *kurstaki* strain HD-1. The modification was required to improve gene expression in plants. The firm states that the amino acid sequence of the expression product is identical to that of the native protein. Inserted between the *cryIA(b)* gene and its E35S promoter is the intron from the corn *hsp70* gene. The intron was introduced to increase transcription levels of *cryIA(b)*. The NOS 3' nontranslated sequence served to terminate transcription of *cryIA(b)* gene, and to direct mRNA polyadenylation.

The *CP4 EPSPS* gene from PV-ZMGT10 is utilized as a selectable marker for transformed MON809 corn plants. The gene was isolated from *Agrobacterium* strain CP4. To exhibit herbicide tolerance, the CP4 EPSPS protein must be translocated into the chloroplast. To achieve this, the chloroplast transit peptide coding sequence (CTP2) from the *Arabidopsis thaliana* EPSPS was fused to the N-terminus of the CP4 protein. The transit peptide is cleaved from the enzyme after arrival in the chloroplast. In addition to *CP4 EPSPS*, PV-ZMGT10 also contains the *gox* gene, isolated from *Ochrobactrum anthropi* (formerly *Achromobacter*) strain LBAA, as another source of selectable, herbicide tolerance. This gene codes for glyphosate oxidoreductase, an enzyme that degrades glyphosate. The gox enzyme also requires translocation into the chloroplast for activity.

To accomplish the translocation, another transit peptide, CTP1, derived from the ribulose-1,5-bisphosphate carboxylase (SSU1A) gene from *Arabidopsis thaliana* was used. The *hsp70* intron was used to increase transcription levels of both herbicide tolerance genes.

However, Monsanto reports that a partial, rearranged *gox* gene is present in MON809 corn, and the enzyme is not expressed as determined by western blot and ELISA analyses of grain, leaf and whole plant tissues.

Expressed Proteins

Monsanto reports that both expression products (cryIA(b) and CP4 EPSPS) are only a small portion of MON809 plant protein. In MON809, cryIA(b) makes up 1.63, 0.55, and 1.23 µg/g fresh tissue, while CP4 EPSPS represents 21.68, 9.41, and 1.6 µg/g of leaf, grain, and whole plant tissue, respectively. As expected, only cryIA(b) was detected in the leaf, grain, and whole plant tissue of MON810 (9.35, 0.31, 4.15 µg/g fresh tissue, respectively). The firm reports that cryIA(b) is not present in MON809 pollen, but was detected in pollen from MON810 at the level of 0.09 µg/g.

Regulatory Considerations

Monsanto states that the cryIA(b) protein present in MON809 and MON810 is identical to that present in nature and commercial microbial preparations approved by the Environmental Protection Agency (EPA). The safe use of insecticidal proteins as pesticides and the use of selectable markers as pesticidal inert ingredients in the development of insect resistant plant varieties are regulated by EPA under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and the Federal Food, Drug, and Cosmetic Act (FFDCA). Therefore, we have not addressed the safe use of cryIA(b) as a pesticide or the safe use of CP4 EPSPS and gox as pesticidal inert ingredients.

Nutritional Assessment

Grain

Based on the nature of the genetic modification, it was expected that MON809 and MON810 corn would not materially differ in composition from other corn varieties. To confirm this expectation, Monsanto analyzed the nutrient composition of grain obtained from MON809 and MON810 corn and a comparable control, MON818, by standard methods for protein, fat, ash, carbohydrates, moisture, calories, amino acid profile, and fatty acid composition. The fatty acids analyzed were linoleic, oleic, palmitic, stearic, and linolenic. Grain samples were also analyzed for calcium and phosphorus levels. Monsanto indicates that there were some statistically significant differences in the measured analytes, but the changes were considered negligible. Monsanto concludes that MON809 and MON810 are similar in composition to the control, MON818, and other commercial corn varieties.

Vegetative Tissues

Compositional analyses of MON810 green chop (silage) included protein, total fat, ash, carbohydrates, neutral detergent fiber, acid detergent fiber, and dry matter in three field trials. Forage composition information is not available for MON809. Monsanto indicates that forage produced by MON810 corn is similar to that of the control line, MON820. Monsanto concludes this section by stating that the absence of unintended effects in MON809 and MON810 corn is demonstrated by the safety of the host, corn and compositional analyses.

Conclusions

Monsanto has concluded that "sales and consumption of corn grain, fodder, and silage derived from these corn lines and all progenies derived from crosses with these lines and introduced varieties would be fully consistent with the Agency's Food Policy, the Federal Food, Drug, and Cosmetic Act, and current practices for the development and introduction of new corn varieties." Based on the information Monsanto has presented, we have no further questions about corn products containing the MON810 transformation event or grain obtained from lines containing the MON809 event. However, no information was presented concerning the composition of forage obtained from corn plants containing the MON809 transformation event. At this time, the Agency considers the consultation on corn lines containing transformation events MON809 and MON810 to be complete.

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