

NOTE TO THE FILE

BNF0071

October 09, 2000

Subject: Monsanto Roundup Ready® Corn line NK603.

Keywords:

Corn, Roundup Ready®, *Zea mays*, Glyphosate (N-phosphonomethyl-glycine), 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), CP4 EPSPS gene, *Agrobacterium* sp. CP4 strain, Herbicide-Tolerant, Glyphosate-Tolerant.

Background:

In a submission dated February 28, 2000, Monsanto provided summary information to support the safety and nutritional assessment of their Glyphosate-tolerant (Roundup Ready®) corn, NK603 line. In 1998, Monsanto completed a consultation with the FDA for another Roundup Ready® transgenic GA21 corn (for detailed information refer to BNF 0051).

Intended effect and food/feed use

The intended effect of the NK603 genetic modification is to confer tolerance to the herbicidal compound N-phosphonomethyl-glycine, the active moiety of glyphosate (Roundup®). In non-transgenic plants, glyphosate binds to the plant 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) enzyme and blocks the biosynthesis of aromatic amino acids. The transgenic NK603 corn developed by Monsanto, contains the EPSPS gene from the *Agrobacterium* sp. strain CP4. The CP4 EPSPS enzyme has a reduced affinity for glyphosate when compared to the native corn EPSPS enzyme. As a result, corn plants expressing the CP4 EPSPS enzyme become tolerant to glyphosate.

Molecular alterations and characterization

Monsanto transformed embryonic cells derived from corn AW x CW inbred line using the microprojectile bombardment technique to produce the transformation event NK603 corn. The transformation vector PV-ZGMT32 consists of two adjacent plant gene expression cassettes, each containing a single copy of the CP4-EPSPS gene fused to chloroplast transit peptide (CTP) sequences based on sequences isolated from *Arabidopsis thaliana* EPSPS. The first gene cassette contains the CP4 EPSPS coding sequence under the control of the 5' end of the rice actin sequence (*ract1*) containing the promoter and the first intron upstream of the CTP sequence. The second gene cassette contains the CP4 EPSPS gene coding sequence under the control of the enhanced CaMV 35S promoter fused to the 0.8 kb intron sequence from the gene of maize heat shock 70 protein. Both gene cassettes contain the 3'-untranslated region of the nopaline synthase gene (*nos 3'*) from the *Agrobacterium tumefaciens* T-DNA to terminate transcription of the CP4

EPSPS. The plasmid also contains an origin of replication (*ori*) required for replication of the plasmid in *E.coli*, and the *nptII* bacterial selectable marker gene encoding kanamycin resistance to ensure the selection of bacteria containing the plasmid. The plasmid fragment utilized for transformation of the Roundup Ready® NK603 corn does not contain the *nptII* marker gene or the *ori* sequence.

Monsanto performed segregation study for nine generations of line NK603 progeny. These generations include: BC0F1 (derived from crossing the R0 with the public inbred line "B73"), BC1F1 (derived from crossing the BC0F1 plants with B73), BC2F1, BC3F1, BC4F1, BC2F2 (derived from selfing individual BC2F1 plants), BC2F3 (derived from selfing individual BC2F2 plants), and BC4F3 generation. According to Monsanto, all generations segregated as expected for a single insertion locus in a 1:1 segregation ratio of herbicide tolerant: herbicide susceptible plants, except for the BC2F1 generation. The BC2F1 generation had a higher than predicted number of positive (CP4-gene-containing) plants due to gamete selection as a result of high application rates of glyphosate in the generation prior to the BC2F1 (i.e., BC1F1). The stability of the insert has been demonstrated through six generations of crossing and three generations of self pollination.

To determine the nature and number of EPSPS gene insertions in the transformation event NK603, the notifier conducted Southern hybridization of genomic DNA from transgenic NK603 plants, non-transgenic B73 plants mixed with plasmid PV-ZMGT32, and from non-transgenic B73 corn. Data showed that the NK603 corn contains a single DNA insertion of the two intact CP4 EPSPS gene cassettes. The insertion also includes a non-functional, inversely linked 217-bp fragment of the enhancer region of the rice actin promoter at the 3' end. According to Monsanto, the genome of corn line NK603 does not contain any detectable plasmid backbone. The 5' and 3' ends of the corn NK603 insert were also verified by polymerase chain reaction (PCR) and DNA sequencing.

Monsanto performed Southern analysis of genomic DNA of transgenic plants from the F1 generation (the progeny of the R0 back-crossing) and the fifth generation of back-crossing (BC5F1), non-transgenic B73, and from non-transgenic B73 plants mixed with plasmid PV-ZMGT32. The banding pattern ("fingerprint") was identical across generations and genotypes confirming the genetic stability of the inserted DNA in NK603 corn lines.

Expression of CP4 EPSPS gene in transformation event NK603

The notifier conducted a double antibody sandwich enzyme linked immunosorbent assay (ELISA) to measure the level of expression of the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) in forage and grain of transgenic NK603 and non-transgenic parental control line (LH82 x B73). The samples were collected from the plants of six non-replicated and two replicated field sites. According to Monsanto, the EPSPS protein was expressed in transgenic plants at very low but similar levels within sites or across geographically dispersed sites. The levels of CP4 EPSPS in control forage and grain were below the limit of quantitation.

Monsanto performed Western blot analysis to assess the equivalence of CP4 EPSPS protein produced in NK603 plants with the microbially-produced CP4 protein. Equivalence was confirmed by identical apparent molecular weights (~ 47 KDa) and immunological properties when detected using antibodies specific for CP4 EPSPS protein.

Safety of CP4 EPSPS gene in transformation event NK603

Monsanto presented information and published data to support the safety of the CP4 EPSPS protein for human and animal consumption. These data include

- 1) history of safe consumption of the CP4 EPSPS protein expressed in Roundup Ready® soybean,
- 2) lack of acute toxicity as determined by a mouse gavage study,
- 3) rapid digestion in simulated gastric and intestinal fluid
- 4) lack of amino acid sequence homology with known protein toxins, and
- 5) probable lack of allergenic potential for the gene product, based on lack of immunologically relevant sequence similarity to known allergens, and its low concentration in corn in comparison to normal levels of known food allergens in the diet.
- 6) lack of significant difference in the antinutrient content between NK603 corn and the control variety

Compositional profile

Monsanto presented data that allowed the firm to conclude that the Roundup Ready® corn line NK603 is not materially different in any meaningful way from corn varieties now being sold except for the tolerance to Roundup® herbicide. Monsanto presented the results of extensive compositional analyses (fifty one compositional components) to demonstrate that the levels of important components of corn grain and forage are similar in the transgenic NK603 line and non-transgenic control line. Samples of grain and forage were collected from several field trials. The components that Monsanto measured included:

- 1) grain: proximates (protein, fat, carbohydrate, ash and moisture), acid detergent and neutral detergent fiber, fatty acids, amino acids, minerals (copper, iron, manganese, calcium, magnesium, phosphorus, potassium, sodium, and zinc), vitamin E, trypsin inhibitor, and phytic acid.
- 2) green forage: moisture, proximates and acid detergent and neutral detergent fiber.

Monsanto reported that for the grain there was small but significant differences in the amino acids arginine, cystine and phenylalanine, and for the minerals calcium, magnesium and phosphorus. These nutrient levels are within the literature range, or in the case of calcium within the range of values reported in previous BNFs. Monsanto also reported small but significant differences in the fatty acids palmitic, stearic, oleic and icosanoic acids. Again, Monsanto noted that the levels of these fatty acids are within the reported literature range.

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The results for the forage indicate small but significant differences in carbohydrate, protein and moisture contents in one of three individual trials for the NK630 vs. control, but the values for NK630 fall within the literature ranges. Difference in moisture content is due to the time of harvest, and is not considered a key nutrient.

Conclusions

Monsanto has concluded that its transgenic NK603 corn is not materially different in terms of food safety and nutritional profile from non- transgenic corn hybrids currently on the market. At this time, based on Monsanto's description of its data and analysis, the agency considers Monsanto's consultation on the Roundup Ready® NK603 corn line to be complete.

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