Safety Assessment of Roundup Ready® Corn Event NK603

Executive Summary

Using modern biotechnology, Monsanto Company has developed Roundup Ready® corn plants that confer tolerance to glyphosate, the active ingredient in Roundup® agricultural herbicides, by the production of the glyphosate-tolerant CP4 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) proteins. Glyphosate kills plants by inhibiting the enzyme EPSPS. This enzyme catalyzes a critical step in the shikimic acid pathway for the biosynthesis of aromatic amino acids in plants and microorganisms, and its inhibition leads to the lack of growth in plants. The CP4 EPSPS proteins have a low affinity for glyphosate compared to the wild-type EPSPS enzyme. Thus, when corn plants expressing the CP4 EPSPS proteins are treated with glyphosate, the plants continue to grow. The continued action of the tolerant CP4 EPSPS enzyme provides the plant's need for aromatic acids. Aromatic amino acid biosynthesis is not present in animals. This explains the selective activity in plants and contributes to the low mammalian toxicity of glyphosate. Two copies of the *cp4 epsps* gene were introduced into the corn genome to produce Roundup Ready corn event NK603. The *cp4 epsps* gene derived from the common soil bacterium *Agrobacterium* sp. strain *CP4* encodes for the naturally glyphosate-tolerant EPSPS protein.

The food and feed safety of corn event NK603 was established based upon: the evaluation of CP4 EPSPS activity and homology to EPSPS proteins present in a diversity of plants, including those used for foods; the low dietary exposure to CP4 EPSPS; the rapid digestibility of CP4 EPSPS; and the lack of toxicity or allergenicity of EPSPSs generally and by safety studies of the expressed CP4 EPSPS proteins. The equivalence of corn event NK603 compared to conventional corn was demonstrated by analyses of key nutrients including protein, fat, carbohydrates, moisture, amino acids, fatty acids, and minerals. Nutritional equivalence of corn event NK603 compared to conventional corn was confirmed by evaluation of the feed performance in broiler chickens and a rat feeding study, which included clinical and histological evaluations. The environmental impact of Roundup Ready corn is comparable to conventional corn. Glyphosate-tolerant volunteer corn is infrequent and easily managed in the farmer's field. The results of all these studies demonstrate that corn event NK603 is comparable to traditional corn with respect to food, feed and environmental safety.

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Introduction

Using the methods of modern biotechnology, Monsanto Company has developed Roundup Ready® corn hybrids that confer tolerance to glyphosate, the active ingredient in Roundup® agricultural herbicides, by the production of 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) proteins that naturally confer tolerance to glyphosate. The EPSPS enzyme is present in the shikimic acid pathway for the biosynthesis of aromatic amino acids in plants and microorganisms. Inhibition of this enzyme by glyphosate leads to a reduction of aromatic amino acids and lack of growth in plants. The aromatic amino acid biosynthetic pathway is not present in mammalian, avian or aquatic animals. This explains the selective activity in plants and contributes to the low risk to human health and the environment from the use of glyphosate according to label directions.

Roundup Ready corn offers growers an additional tool for improved weed control. The use of Roundup Ready corn provides:

- *Broad-spectrum weed control.* Roundup agricultural herbicides control both broadleaf weeds and grasses, including difficult to control weed species (Franz *et al.*, 1997).
- *Excellent crop safety.* When used according to label directions, Roundup agricultural herbicides control weeds without injury to the Roundup Ready corn.
- *Favorable environmental properties.* Roundup agricultural herbicides have been used for almost 30 years in various applications. Glyphosate, the active ingredient in Roundup agricultural herbicides, has favorable environmental characteristics, including that it binds tightly to soil, making it unlikely to move to groundwater or reach non-target plants, and that it degrades over time into naturally occurring materials. In addition, glyphosate will not cause unreasonable adverse effects to the environment under normal use conditions (US EPA, 1993; WHO, 1994; Geisy *et al*., 2000).
- *Flexibility in treating for weed control.* Since Roundup agricultural herbicides are applied onto the foliage of weeds after crop emergence, applications are only necessary if weed infestation reaches the threshold level for yield reductions.
- *High compatibility with Integrated Pest Management and soil conservation techniques.* Benefits of conservation tillage include improved soil quality, improved water infiltration, reduced soil erosion and sedimentation of water resources, reduced runoff of nutrients and pesticides to surface water, improved wildlife habitat, increased carbon retention in soil, reduced fuel usage, and use of sustainable agricultural practices (Warburton and Klimstra, 1984; Edwards *et al.,* 1988; Hebblethwaite, 1995; Reicosky, 1995; Reicosky and Lindstrom, 1995; Keeling *et al*., 1998; CTIC, 1998; CTIC, 2000).
- *Cost effective weed control.* The cost of weed control with Roundup agricultural herbicides is competitive with the cost of alternative weed control options,

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especially in view of the high weed control efficacy of Roundup. Both large and small-scale farmers benefit equally from use of this technology.

- *Provides an additional herbicidal mode of action for in-season corn weed control.* Roundup agricultural herbicides can only be used in pre-plant applications (in all but a few pre-harvest uses) without the Roundup Ready genetic modification in the crop.
- *Use of an herbicide with low risk to human health.* Under present conditions of use, Roundup agricultural herbicides will not cause unreasonable adverse effects on human health (U.S. EPA, 1993; WHO, 1994; Williams *et al*., 2000). Glyphosate has been classified by the U.S. EPA as Category E (evidence of non-carcinogenicity for humans) (U.S. EPA, 1992). Additionally, the World Health Organization stated in 1994 that glyphosate is not carcinogenic, mutagenic, or teratogenic (WHO, 1994).

The first Roundup Ready corn event (GA21) was commercialized in the U.S. in 1998 and in Canada in 1999. Extensive testing demonstrated that Roundup Ready corn event GA21 is equivalent to conventionally produced corn in safety, nutrition, composition and environmental impact (Sidhu *et al*., 2000). The Roundup Ready corn containing the GA21 event uses the mEPSPS protein for conferring tolerance to glyphosate. In contrast, corn event NK603 contains the CP4 EPSPS proteins. The new product, containing event NK603 was commercialized in both the U.S. and Canada in 2001. In field trials, corn event NK603 was selected based upon agronomic parameters and tolerance to glyphosate. These trials, established since 1997 across a broad geographic range of environments, have shown no phenotypic differences, except for tolerance of glyphosate, demonstrating that corn event NK603 and its progeny are no different from corn varieties developed through traditional breeding methods, except for the introduced trait. The use of Roundup agricultural herbicides in Roundup Ready corn provides growers with options for in-season weed control and the public with a number of environmental benefits.

This summary provides an assessment of the human health safety of the CP4 EPSPS proteins present in the NK603 corn transformation event based upon the characterization and mechanism of action of the CP4 EPSPS proteins and their comparability to EPSPS enzymes commonly found in a wide variety of food sources, which have a long history of safe use. In addition, the CP4 EPSPS proteins are comparable to the protein found in Roundup Ready soybean and other Roundup Ready crops, which have been safely consumed by humans and animals. Additional studies were conducted and information gathered which supports the safety of the CP4 EPSPS proteins including the: (1) lack of acute toxicity of CP4 EPSPS protein as determined by a mouse gavage study, (2) rapid digestion of CP4 EPSPS proteins in simulated gastric and intestinal fluids, (3) lack of homology of CP4 EPSPS proteins with known protein toxins and (4) lack of allergenic potential of CP4 EPSPS proteins. These data support the assessment of safety of the CP4 EPSPS proteins and, taken together with analyses performed on corn event NK603, demonstrate compositional and nutritional equivalence, and thus support the conclusion that corn event NK603 is as safe and nutritious as conventional corn currently being marketed. These assessments were performed using the principles outlined by independent international scientific bodies such as the Organization for Economic Co-operation and Development (OECD), the

United Nations World Health Organization (WHO) and the Food and Agriculture Organization (FAO) (OECD, 1993; WHO, 1995; WHO/FAO, 1996) and are consistent with country-specific regulations in the U.S., Canada, the EU and other countries.

Molecular Characterization of Corn Event NK603

Corn genetics has been extensively studied for over 100 years. As a result, it is one of the most characterized crop plants. Recently, more complete genetic maps of corn have been developed using molecular genetics. Corn has been used in tissue culture research, molecular marker assisted plant breeding, in the study of transposons for gene tagging and in the study of genetic variability.

The corn event NK603 was developed by introducing two *cp4 epsps* coding sequences into embryogenic corn cells from a proprietary inbred line designated (AW x CW) using the particle acceleration method (Klein *et al.,* 1987; Gordon-Kamm *et al*., 1990). An *Mlu* I restriction fragment that contained two adjacent plant gene expression cassettes each, containing a single copy of the *cp4 epsps* gene (Figure 1), was derived from the plasmid PV-ZMGT32 and was used for transformation. In one cassette, the *cp4 epsps* coding sequence is under the regulation of the rice actin promoter and rice actin intron and contains the *nos* 3' polyadenylation sequence. In the second cassette, the *cp4 epsps* coding sequence is under the regulation of the enhanced 35S promoter from CaMV with an enhanced duplicator region, corn *hsp70* intron and the *nos* 3' polyadenylation sequence. In both plant gene expression cassettes, the *cp4 epsps* coding sequences are fused to chloroplast transit peptide (CTP2) sequences. These are based on sequences isolated from *Arabidopsis thaliana* EPSPS. The CTP targets the CP4 EPSPS proteins to the chloroplast, the location of EPSPS in plants and the site of aromatic amino acid biosynthesis (Kishore and Shah, 1988). CTPs are typically cleaved from the "mature" protein following delivery to the plastid (della-Cioppa *et al.,* 1986).

Following transformation, transformants were selected for their ability to survive and grow in the presence of glyphosate. R0 plants were generated from the embryonic callus by placing the callus on media that stimulates the production of shoots and roots.

Molecular studies demonstrated that Roundup Ready corn plants contain a single insert of DNA. The single insert in corn event NK603 contains:

- a single complete copy of the linear DNA of PV-ZMGT32 used for transformation;
- both CP4 EPSPS gene cassettes, within the single insert, are intact;
- an inversely linked 217 bp piece of DNA containing a portion of the enhancer region of the rice actin promoter at the 3' end of the inserted DNA.

Sequencing of the DNA inserted into corn event NK603 confirmed the molecular details above. Nucleotide sequence of the insert showed that the *cp4 epsps* coding region regulated by the rice actin promoter was as expected. However, the *cp4 epsps* coding

region regulated by the E35S promoter contained two nucleotide changes, one of which results in a change of the amino acid leucine to proline at position 214 in the protein. The CP4 EPSPS protein containing this change is referred to as CP4 EPSPS L214P. The other nucleotide change did not result in an amino acid change.

PCR and DNA sequencing verified the 5' and 3' ends of the insert in corn event NK603. The sequences flanking the insert were confirmed to be native to corn. Expression of the full-length CP4 EPSPS proteins in NK603 plants was confirmed by western blot analysis. As predicted, the two CP4 EPSPS proteins are indistinguishable in western blot analysis with the available polyclonal antibody, since the proteins are essentially identical. These data support the conclusion that only the two full-length CP4 EPSPS proteins are encoded by the insert in event NK603.

In addition to the two complete *cp4 epsps* cassettes, corn event NK603 contains a 217 bp portion of DNA containing part of the enhancer region of the rice actin promoter at the 3' end of the inserted DNA in the inverse direction of the *cp4 epsps* cassettes. RT-PCR analyses were conducted across the 3' junction between the NK603 insert and the adjacent corn genomic DNA sequences to assess transcriptional activity. The results from these analyses demonstrated that mRNA transcription was detected to initiate in either one of the two promoters of the NK603 insert and proceed through the NOS 3' polyadenylation sequence and continue into the corn genomic DNA flanking the 3' end of the insert. This result is not unexpected since the incomplete termination or use of alternative termination sites and resulting production of multiple transcripts has been reported for endogenous genes in plants (Rothnie, 1996; Hunt, 1994; Gallie, 1993) and in corn (Dean *et al.,* 1986). Given the structure of the *cp4 epsps* coding sequence, the surrounding genetic elements and the nature of the plant's protein-producing machinery, any transcripts longer than full-length would either produce a CP4 EPSPS protein longer than the full-length protein or the full-length CP4 EPSPS protein itself. No longer than full-length CP4 EPSPS protein was detected as assessed by western blot analysis. Only the full-length CP4 EPSPS protein was observed. Therefore, it was concluded that only the full-length EPSPS proteins are produced in corn event NK603.

Inheritance of the CP4 EPSPS insert conforms to the expected Mendelian segregation pattern for single genetic loci. The stability of the insert has been demonstrated through more than nine generations of crossing and one generation of self-pollination. In addition, progeny of corn event NK603 have been field tested at multiple sites in the U.S. since 1997 and in the EU since 1999. No instability of the DNA insert has been detected during extensive field testing and commercial production of corn event NK603.

CP4 EPSPS Protein Levels in Roundup Ready Corn Plants

Forage and grain samples collected from field grown corn event NK603 plants were analyzed using enzyme linked immunosorbent assays (ELISA) (Harlow and Lane, 1988) and western blot (Matsudaira, 1987) methods developed and optimized to estimate CP4 EPSPS protein levels in corn forage and grain matrices. Data generated from samples are

presented in Table 1. CP4 EPSPS proteins were detected in event NK603 samples and were not detected, as expected, in the non-modified control line. The mean level of CP4 EPSPS proteins in corn forage was 25.6µg/g tissue on a fresh weight basis. The mean level of CP4 EPSPS proteins in corn grain from event NK603 was 10.9 µg/g tissue. The low levels of CP4 EPSPS protein expression in line NK603 are sufficient to confer tolerance to glyphosate. These reported levels are for the combination of the CP4 EPSPS and CP4 EPSPS L214P proteins, since these proteins are indistinguishable with the antibody used in these assays.

Safety Assessment of CP4 EPSPS Proteins in Corn Event NK603

Safety assessments of the CP4 EPSPS proteins expressed in corn event NK603 include protein characterization (demonstrating the lack of similarity to known allergens and toxins); the long history of safe consumption of similar proteins; digestibility *in vitro*; and the lack of acute oral toxicity in mice of the CP4 EPSPS protein. The CP4 EPSPS protein expressed in corn event NK603 is identical to the protein found in Roundup Ready soybeans, canola, and cotton with a history of safe human and animal consumption. The CP4 EPSPS L214P protein differs by only one amino acid at position 214. Detailed analytical and three-dimensional modeling analyses of the CP4 EPSPS and CP4 EPSPS L214P proteins show that the two proteins are structurally and functionally equivalent. CP4 EPSPS L214P was demonstrated to have equivalent functional activity to CP4 EPSPS, to lack amino acid sequence similarity to toxins and allergens, to be rapidly digested *in vitro* and to have a projected three-dimensional structure essentially indistinguishable from the CP4 EPSPS protein.

CP4 EPSPS and CP4 EPSPS l214P Protein Characterization and History of Consumption in the Context of Food Safety

The CP4 EPSPS proteins produced in Roundup Ready corn are functionally similar to a diverse family of EPSPS proteins present in food and feed derived from plant and microbial sources (Levin and Sprinson, 1964; Harrison *et al.*, 1996). The EPSPS protein is required for the production of aromatic amino acids. The structural relationship between CP4 EPSPS and CP4 EPSPS L214P and other EPSPS proteins found in food is demonstrated by comparison of the amino acid sequences with conserved identity of the active site residues, and the expected conserved three-dimensional structure based on similarity of the amino acid sequences. The structural and functional equivalence of CP4 EPSPS and CP4 EPSPS L214P were based on the demonstration that proline residues naturally occur near position 214 in extant EPSPS proteins; modeling using the known X-ray crystal structure of CP4 EPSPS, which showed that the L214P substitution does not alter the predicted secondary and tertiary structure of CP4 EPSPS; equivalent enzymatic activity for CP4 EPSPS and CP4 EPSPS L214P; knowledge that the variable loop region containing the proline substitution is not relevant to the enzymatic activity of EPSPSs generally; and the fact that the CP4 EPSPS protein domain containing the proline substitution is highly heterogenous in all known EPSPS proteins.

Assessment of Sequence Similarity of CP4 EPSPS and CP4 EPSPS L214P Proteins to Known Protein Toxins

Potential toxicity effects of proteins can be deduced by comparisons between the amino acid sequence of the introduced protein to known protein toxins. Homologous proteins derived from a common ancestor will have highly similar amino acid sequences, are structurally similar and often share common function. Therefore, the first step to assess potential toxicity of proteins is to evaluate sequence similarity to known protein toxins. Homology is determined by comparing the degree of amino acid similarity between proteins using published criteria (Doolittle, 1990). When homology to known toxins is identified, the structural and functional implications of the homology can be assessed by experimentation. When no homology exists, general oral toxicity screening will be employed as below. Bioinformatics assessments of CP4 EPSPS and CP4 EPSPS L214P proteins show that these proteins are similar only to proteins of the EPSPS gene family, and are not similar to toxins or other pharmacologically active proteins contained in the PIR, EMBL, SwissProt and GenBank protein sequence databases.

Digestion of CP4 EPSPS and CP4 EPSPS L214P Proteins in Simulated Gastric and Intestinal Fluids

In vitro, simulated mammalian gastric and intestinal digestive mixtures were used to assess the susceptibility of the CP4 EPSPS and CP4 EPSPS L214P proteins to proteolytic digestion. Rapid degradation of the proteins correlates with limited exposure to the gastrointestinal tract and little likelihood that the CP4 EPSPS proteins would be food allergens. The method of preparation of the simulated digestion solutions used is described in the United States Pharmacopeia (1995).

The CP4 EPSPS protein was shown to be rapidly degraded by the components of the *in vitro* digestive system (Harrison *et al.*, 1996). Western blot analysis demonstrated a half-life for CP4 EPSPS protein of less than 15 seconds in the simulated gastric system and less than 10 minutes in the simulated intestinal system. Similarly, the CP4 EPSPS L214P protein was also shown to have a half-life of less than 15 seconds in simulated gastric fluid. If the CP4 EPSPS proteins were to survive the gastric system, they would be rapidly degraded in the intestine. Rapidly digested proteins represent a minimal risk of conferring novel toxicity or allergy comparable to other safe dietary proteins (Astwood *et al*., 1996; Astwood and Fuchs, 2000).

Assessment of Acute Oral Toxicity of CP4 EPSPS Protein in Mice

Few proteins are toxic when ingested and those that are toxic typically act in an acute manner (Sjoblad *et al.*, 1992). Thus, acute administration to mice was considered appropriate to assess any potential toxicity associated with the CP4 EPSPS protein (Harrison *et al.*, 1996). There were no treatment-related adverse effects in mice administered CP4 EPSPS protein by oral gavage at dosages up to 572 mg/kg. Results from this study demonstrated that the CP4 EPSPS protein is not acutely toxic to mammals. This result was expected since CP4 EPSPS is readily digested in gastric and intestinal fluids *in vitro* and is from a family of proteins with a history of safe consumption.

Assessment of Potential Allergenicity of CP4 EPSPS and CP4 EPSPS L214P Proteins

It is recognized that most food allergens are naturally occurring proteins. Although large quantities of a range of proteins are consumed in human diets each day, rarely do any of these tens of thousands of proteins elicit an allergenic response (Taylor, 1992). While there are no predictive bioassays available to assess the allergenic potential of proteins in humans (U.S. FDA, 1992), the physicochemical and human exposure profile of the protein provides a basis for assessing potential allergenicity by comparing it to known protein allergens. Thus, important considerations contributing to the allergenicity of proteins ingested orally includes exposure and an assessment of the factors that contribute to exposure, such as stability to digestion, prevalence in the food, and consumption pattern (amount) of the specific food (Metcalfe *et al.,* 1996; Kimber *et al.,* 1999).

A key parameter contributing to the systemic allergenicity of certain food proteins appears to be stability to gastrointestinal digestion, especially stability to acid proteases like pepsin found in the stomach (Astwood *et al.,* 1996; Astwood and Fuchs, 1996; Fuchs and Astwood, 1996; FAO, 1995; Kimber *et al.,* 1999). Important protein allergens tend to be stable to peptic digestion and the acidic conditions of the stomach if they are to reach the intestinal mucosa where an immune response can be initiated. As noted above, the *in vitro* assessment of the digestibility of the CP4 EPSPS and CP4 EPSPS L214P proteins indicates that these proteins are readily digested.

Another significant factor contributing to the allergenicity of certain food proteins is their high concentration in foods (Taylor *et al.,* 1987; Taylor, 1992; Fuchs and Astwood, 1996). Most allergens are present as major protein components in the specific food, representing from 2-3% up to 80% of total protein (Fuchs and Astwood, 1996). The CP4 EPSPS proteins are present at extremely low levels -- approximately 0.01% of the total protein found in the grain of Roundup Ready corn.

It is also important to establish that the proteins do not represent a previously described allergen and do not share potentially cross-reactive amino acid sequence segments or structure with a known allergen. An efficient way to assess whether the added proteins are allergens or are likely to contain cross-reactive structures is to compare the amino acid sequence with that of all known allergens. A database of protein sequences associated with allergy and coeliac disease has been assembled from publicly available genetic databases (GenBank, EMBL, PIR and SwissProt). The amino acid sequences of the CP4 EPSPS and CP4 EPSPS L214P proteins were compared to these sequences. The CP4 EPSPS and CP4 EPSPS L214P proteins do not share any meaningful amino acid sequence similarity with known allergens (Astwood *et al.*, 1996).

In summary, the known function and ubiquity of EPSPS proteins and direct studies of the CP4 EPSPS proteins demonstrate that these proteins do not represent a risk in the food supply. Results show that there were no indications of toxicity in mice administered CP4 EPSPS protein by oral gavage. This lack of toxicity was expected based on the rapid degradation of the CP4 EPSPS proteins and loss of enzymatic activity in simulated human gastric and intestinal fluids. In addition, the CP4 EPSPS proteins are not homologous to known protein toxins or allergens

and are present at very low levels in Roundup Ready corn. Furthermore, these proteins are from a family of proteins with a long history of safe consumption. And finally, the CP4 EPSPS protein expressed in corn event NK603 has a history of safe consumption due to the use of Roundup Ready soybean expressing the same protein for glyphosate tolerance. CP4 EPSPS L214P was demonstrated to have equivalent functional activity to CP4 EPSPS, lack amino acid sequence similarity to toxins and allergens, and to be rapidly digested *in vitro*. Based on these data, CP4 EPSPS L214P was determined to be structurally and functionally equivalent to the CP4 EPSPS protein and thus is safe for human and animal consumption.

Compositional Analysis and Nutritional Assessment of Roundup Ready Corn

Although an ideal source of energy, relatively low levels of whole kernel or processed corn are consumed by humans worldwide when compared to corn-based food ingredients (Hodge, 1982 and Watson, 1988). Corn is an excellent raw material for the manufacture of starch, not only because of price and availability, but also because the starch is easily recovered in high yield and purity (Anderson and Watson, 1982). Nearly 25% of corn starch is sold as starch products; more than 75% of the starch is converted to a variety of sweetener and fermentation products, including high fructose corn syrup and ethanol (Watson, 1988; National Corn Growers Association, 1995). Additionally, corn oil is commercially processed from the germ and accounts for approximately nine percent of domestic vegetable oil production (Orthoefer and Sinram, 1987). Each of these materials is a component of many foods, including bakery and dairy goods, beverages, confections and meat products.

Feed for animals is by far the largest use of corn in the United States, with more than half (50-60%) of annual production fed to cattle, chickens and swine (Hodge, 1982; U.S. Feed Grains Council, 1999; Watson, 1988). Corn is readily consumed by livestock and, because of its high starch and low fiber content, is one of the most concentrated sources of energy, containing more total digestible nutrients than any other feed grain.

Compositional Analysis

Compostional analyses are a critical component of the safety assessment process. To assess whether the composition of Roundup Ready corn is comparable to conventional corn present in the marketplace – with the exception of the introduced trait – corn grain and forage composition were measured. Compositional analyses were conducted on the key corn tissues, grain and forage, produced in in Kansas, Iowa, Illinois, Indiana, and Ohio in 1998 and in trials in Italy and France in 1999. Grain and forage samples were taken from plants of the corn event NK603 and the non-modified control both years. In the E.U. field trials, reference grain and forage samples also included 19 conventional, commercial hybrids (five hybrids per site with one hybrid planted at two sites). The NK603 plants were treated with Roundup Ultra® herbicide. Fifty-one different compositional components were evaluated. These analyses included:

- *Proximates*: protein, ash, fat, carbohydrates, and moisture in forage and grain (Tables 2 and 3);
- *Fiber*: acid detergent fiber (ADF), neutral detergent fiber (NDF) in forage and grain (Tables 2 and 3);
- *Minerals*: phosphorus, calcium, potassium, magnesium, copper, iron, manganese and zinc in grain (Tables 2 and 3);
- *Amino acid composition*: each amino acid expressed as percent of total protein in grain (Table 4);
- *Fatty acids*: percentage of individual fatty acids in grain (Table 5);
- *Vitamin E, phytic acid and trypsin inhibitor* in grain (Table 6);
- *Secondary metabolites*: ferulic acid, p-courmaric acid, and raffinose (Table 6).

Statistical analyses were conducted on the data using a mixed model analysis of variance for a combination of all sites for 1998 and a combination of two sites with a randomized complete block design for the 1999 studies. There were a total of 51 components evaluated (seven in forage and 44 in grain) both in 1998 and 1999. The 44 components in grain resulted from the difference between the initial 59 components minus 16 components that were excluded because their levels were below the level of quantitation. Compositional data from the commercial reference lines in the 1999 study were not included in the statistical analysis. However, population tolerance intervals were determined for each component by calculating the range of the reference values and the variation among the values to estimate the upper and lower boundaries of the entire population. For each compositional component, tolerance intervals were calculated that are expected to contain, with 95% confidence, 99% of the values expressed in the population of commercial lines.

Compositional analysis results generated from nine field sites over a period of two years show that the grain and forage of corn event NK603 are comparable in their composition to those of the control corn and to conventional corn. At the 5% level of significance, one of twenty comparisons between the corn event NK603 and the control corn is expected to be significantly different statistically by chance alone. The use of multi-year data and incorporation of reference corn into field trials suggests that the few statistically significant differences observed are most likely due to random chance and unlikely to be of biological relevance. Moreover, the composition of corn event NK603 was shown to fall within the 99% tolerance interval for components in nineteen non-transgenic commercial corn varieties grown as part of the 1999 field trials in Europe, and also fell within the ranges of values reported for non-transgenic corn in the literature as well as in historical data. These latter comparisons are important and relevant because it is well recognized that the composition of any crop, including corn, varies as a result of many factors, including variety, growing conditions and methods of analysis. The values for components in corn event NK603 all fell within the range of natural variability found in non-transgenic corn.

The analysis of the data reported herein illustrates that the tolerance interval is a useful statistical tool that can account for extant natural variability in any measured parameter, especially food and feed nutritional profiles as measured by biochemical composition. From the perspective of safety

assessment, the biochemical sampling described in this study provides a robust measure of unexpected effects due to the insertion of the *cp4 epsps* gene into the corn genome. These nutritional analyses show that the genetic enhancement of conventional corn with corn event NK603 did not produce significant changes in 51 biologically and nutritionally important components. The values for all the compositional components assessed were either comparable to those in grain and forage of the control line, within published literature ranges for non-transgenic commercial corn hybrids, (Jugenheimer, 1976; Watson, 1982; Watson, 1987), within the tolerance interval determined for commercial varieties evaluated in the 1999 field trials, or within the range of historical conventional control values determined from previous studies. Based on the principle of substantial equivalence as articulated by the World Health Organization, Organization for Economic Cooperation and Development as well as the United Nations Food and Agriculture Organization, these data support the conclusion that corn event NK603 is as safe and nutritious as conventional varieties of corn in the market in the today.

Nutritional Assessment and Toxicological Assessment of Grain

From a nutritional perspective, the single most informative measure of adverse effects (*i.e.*, pleiotropy) due to the insertion and/or expression of introduced genes, are measures of growth performance of animals fed diets which incorporate grain or grain fractions. Two key animal feeding studies have been completed using diets incorporating raw corn grain or ground grain containing corn event NK603. The animal feeding studies included a 42-day chicken study and a 90-day rat study. These studies confirm the nutritional and toxicological equivalence of corn containing event NK603 to conventionally bred corn when used as animal feed.

Broilers are highly sensitive to small nutrient changes within their diets because of their extremely rapid growth. Within the poultry studies conducted by Monsanto, the power of the test is sufficient to detect 2-4% differences in the mean of the test parameter. In a full life study of 42 days, broilers increase in weight by some 50-fold, creating a very sensitive indicator of nutritional changes in the feed. A 42-day chicken study was undertaken to compare the nutritional value of corn containing the NK603 corn event to the non-modified control and six non-modified commercially available corn lines fed to Ross x Ross broiler chickens. The diets were formulated based on the individual nutrient analyses for the grain from each test, control and commercial line to meet nutritional recommendations (National Research Council, 1994). From days 1-20, chickens were fed a starter diet containing approximately 55% w/w corn. From days 20-42, chickens were fed a grower/finisher diet containing approximately 60% w/w corn. These dietary corn concentrations are within the range used by commercial poultry growers in the United States.

Results of this study have been published (Taylor *et al.*, 2001). Results from all groups were compared using conventional statistical methods to detect differences between groups in measured parameters. All performance parameters measured were similar ($P>0.05$) across the broilers fed diets containing NK603 corn, non-modified corn and the six commercial corn lines. Live weight at day 0, live weight at day 42, total feed intake and feed efficiency were similar across all treatments. Broilers fed diets containing NK603 corn had a similar adjusted feed

efficiency as the non-modified control and one of the five commercial reference lines (Table 7). Diets containing the other four reference lines had slightly poorer adjusted feed efficencies than corn event NK603 (on average, 2.3% poorer than NK603). Live weight, chill weight, breast meat, thighs, drums and wings were not affected by diets ($P<0.05$). Fat pad and breast meat weights of the corn event NK603 birds were significantly lower than the non-modified line and all or some of the reference lines. However, these values were within the range of literature values reported in studies using the Ross x Ross strain of broilers (Esteve-Garcia and Llarudado, 1997; Grey *et al*., 1983; Kidd and Kerr, 1997; Lei and Van Beek, 1997; Smith *et al*., 1998; Farran *et al*., 2000; and Peak *et al.*, 2000).

A 90-day study in rats compared the impact of diets containing corn event NK603 grain to its non-modified control and six non-modified commerical corn hybrids of divergent genetic backgrounds. Rats fed diets containing corn event NK603 corn grain that had been formulated to meet specificiations for certified diets had similar responses to rats fed the non-modified control and commercial corn grain diets. Rats were fed one of the following diets for 13 weeks: diets containing 11 or 33% (w/w) corn event NK603 or control corn grain; or diets containing 33% (w/w) reference control grain. Moreover, clinical parameters (hematology, clinical chemistry, urinalyis) and gross and microscopic pathology findings in the animals fed diets of corn event NK603 compared favorably to rats in the non-modified control and commercial corn grain populations. The results of this study confirm the comparability of corn event NK603 to the non-modified control and the commercial grain diets.

The absence of biologically relevant differences in all growth, feed efficiency, histological and clinical parameters studied in either chickens or rats when compared to non-modified control and commercial corn grain confirms the compositional and nutritional equivalence of corn containing the event NK603, the absence of any significant pleiotropic or unintended effects and the absence of toxicity of the CP4 EPSPS and CP4 EPSPS L214P proteins. Both the chicken and rat feeding studies confirm the conclusions of human and animal health safety of corn event NK603 and the nutritional equivalence of corn event NK603 to conventional corn varieties.

Environmental Assessment

Environmental assessment of plants enhanced through modern biotechnology is an important evaluation that occurs prior to commercialization. The approach taken was to evaluate corn event NK603 for the potential to have increased weediness properties and altered interactions with known pests and non-target organisms. In addition, potentially harmful effects on biodiversity due to outcrossing to wild relatives were assessed. Information on the biology and agronomic properties of conventional corn serves as the reference point for assessing whether the modified plant has been meaningfully changed. Toxicity and nutrition studies as well as information about the phenotype conferred by the introduced proteins are key information used in an assessment of the potential environmental impact of the trait.

Corn

Corn (*Zea mays* L.), or maize, is one of the few major crop species indigenous to the Western Hemisphere. Corn is grown in nearly all areas of the world and ranks third behind rice (*Oryza sativa* L.) and wheat (*Triticum* sp.) in total production. The origin of corn has been studied extensively, and it seems its probable domestication was in southern Mexico more than 7,000 - 10,000 years ago. Several hypotheses for the origin and parentage of corn have been advanced (Mangelsdorf, 1974). Today, corn is highly domesticated and, since it could not persist without human intervention, it is not considered weedy.

Evidence has been reported to support the various hypotheses, but the preponderance of evidence supports the hypothesis that corn descended from teosinte (Galinat, 1988), which is a complex of three separate species of *Zea* and two subspecies of *Z. mays* (*Z. diploperennis, Z. perennis*, *Z. luxurians*, *Z. mays ssp. parviglumis*, and *Z. mays ssp. mexicana*). Since the teosinte genome is similar to corn, it is known to form hybrids with corn, and it has several plant morphological traits similar to corn. Unlike corn, teosinte has a more weedy appearance and more tillers than modern corn varieties. One of the more significant features is teosinte's ability to shatter and hence disperse its seed; modern corn does not have this characteristic (Martinez-Soriano and Leal-Klevezas, 2000).

Another major distinguishing difference between corn and teosinte is the female inflorescence, or ear. Modern corn varieties have 1 to 3 lateral branches that terminate in an ear with 8 to 24 kernel rows of 50 seeds, and the ear is enclosed in modified leaves or husks. Teosinte also has lateral branches, but they terminate in two-rowed spikes of perhaps 12 fruit cases, with each fruit case having one seed enclosed by an indurated glume.

Corn has no sexually compatible wild relatives in the U.S. or Europe since teosinte is not present in these regions. The natural distribution of teosinte is limited to the seasonally dry, subtropical zone with summer rain along the western escarpment of Mexico and Guatemala and the Central Plateau of Mexico (Wilkes, 1972). Outside of Central and Southern Mexico, Guatemala and Honduras, there is no meaningful potential for outcrossing to wild, weedy relatives.

Assessment of Agronomic Performance

Corn event NK603 has been tested in the U.S. since 1997 and in the E.U. since 1999. It was first sold commercially in the U.S. and Canada in the spring of 2001. In-crop postemergent application of Roundup agricultural herbicides at labelled rates provides control of a broad range of monocotyledonous and dicotyledonous weed species including foxtail (*Setaria* sp.), panicum (*Panicum* sp.), velvetleaf (*Abutilon theophrasti*), pigweed (*Amaranthus* sp.) and morningglory (*Ipomoea* sp.). Corn event NK603 plants showed excellent crop safety and remained susceptible to labelled rates of a number of alternative herbicides that are labeled for the control of corn.

Evaluations of agronomic characteristics included early plant stand counts, days from planting to 50% pollination, days from planting to 50% silk, ear height, plant height after tasseling, stay

green ratings, number of dropped ears at harvest, grain moisture at harvest, grain test weight at harvest and yield. Statistical evaluation of the data showed that corn event NK603 was equivalent to the non-transgenic control plants except for ear height and days to 50% silking. Corn event NK603 plants had average ear height of 38.8 inches compared to 40.3 inches in the non-transgenic control plants. In addition, corn event NK603 plants had an average number of days to 50% silking of 61.8 days compared to 60.2 days for the non-transgenic control plants. These small differences seen in this early breeding material were considered unlikely to be of biological significance since these were within the range of biological variability for corn. In addition, no differences in the mode or rate of reproduction, corn grain dissemination, or survivability were observed. No differences, except for the tolerance of plants with corn event NK603 to glyphosate, were observed or expected when compared to other corn varieties.

In addition, corn event NK603 was also monitored for its susceptibility to diseases and insects in field trials conducted in the United States over four years. There were no differences in disease severity or insect infestations between corn event NK603 plants and the control plants (USDA, 2000). Since commercialization, corn event NK603 continues to show no unusual plant pest characteristics, nor have any unintended environmental effects been observed that could be attributed to the NK603 insert. As the corn event NK603 has been crossed into an increasing number of existing corn inbreds, agronomic performance has been as expected and tolerance to glyphosate has been uniform and consistent within the new hybrid varieties developed.

Assessment of Effect to Non-Target Organisms

The conventional corn hybrids grown currently are not considered to be harmful to other organisms. There are no indications that Roundup Ready corn is different than other corn in this respect. The CP4 EPSPS proteins, present in Roundup Ready corn at very low levels, have been well characterized and have been demonstrated to be non-toxic in several nutritional and toxicity studies (see above). As mentioned earlier, EPSPS is an enzyme of the shikimate pathway for aromatic amino acid biosynthesis in plants and microorganisms (Levin and Sprinson, 1964; Harrison *et al.,* 1996), and is thus ordinarily present in food derived from plant sources. EPSPSs from a number of bacteria exhibit tolerance to glyphosate (Schulz *et al.*, 1985). CP4 EPSPS thus represents one of many different EPSPSs found in nature. EPSPS is considered to be ubiquitous in nature since it is present in all plants and microorganisms. Therefore, all organisms that presently feed on plants and/or microbes have historically been exposed to EPSPS proteins.

On the basis of the characterization of the introduced proteins and the compositional analyses described above, no specific interactions of Roundup Ready corn with non-target organisms are to be expected, beyond those which occur with other corn hybrids that are treated with other herbicides. The glyphosate tolerance trait is intended to provide protection to the crop when Roundup agricultural herbicides are applied to control competing weeds. Extensive observations in the field have also confirmed that there are no differences between control corn and Roundup Ready corn in phenotype, susceptibility to diseases and predators, or yield, indicating that there is no alteration in the interactions with predatory or beneficial non-target organism.

Impact on Biodiversity

From the extensive testing and commercial experience in the U.S., there is no indication that Roundup Ready corn, compared to other corn, has negative impact on biodiversity. The potential for harm was assessed by considering the intended effects of the genetic modification, as well as the potential for harm resulting from any unintended effects. The intended modification of event NK603 was the expression of CP4 EPSPS proteins conferring tolerance to glyphosate. It has been determined that the CP4 EPSPS proteins are safe for consumption by animals and humans. In addition, agronomic data (discussed above) demonstrate that Roundup Ready corn will behave as other corn hybrids currently used with the exception being tolerance to glyphosate.

The potential for harm resulting from any unintended effects of the modification have been assessed by:

- Observations of the interaction of Roundup Ready corn and other organisms in various environments in the agronomic situation;
- Compositional analyses as indications of unintended modifications in corn grain and forage quality;
- Confirmatory animal feeding studies with raw and processed corn all of which have shown no effect.

Assessment of Resistance to Glyphosate

More than 100 herbicide-resistant weed biotypes have been identified to date; over half of them are resistant to the triazine family of herbicides (Holt and LeBaron, 1990; LeBaron, 1991; Shaner, 1995). Resistance has usually developed because of the selection pressure exerted by the repeated use of herbicides with a single target site and a specific mode of action, long residual activity of the herbicide with the capacity to control weeds year-long, and frequent applications of the same herbicide without rotation to the other herbicides or cultural control practices. Using these criteria, and based on current use data, glyphosate is considered to be a herbicide with a low risk for weed resistance (Benbrook, 1991).

Nonetheless, questions have been raised as to whether the introduction of crops tolerant to a specific herbicide, such as glyphosate, may lead to the occurrence of weeds resistant to that particular herbicide. This concern is based on the assumptions that the use of the herbicide will increase significantly and that it will possibly be used repeatedly in the same location. However, other increases in glyphosate use over the previous years have been more significant than the projected increase associated with the introduction of Roundup Ready crops. Although it cannot be stated that evolution of resistance to glyphosate will not occur, the development of weed resistance to glyphosate is expected to be a very rare event because:

1. Generally, weeds and crop plants are inherently not tolerant to glyphosate, and the long history of extensive use of glyphosate has resulted in few instances of resistant weeds (Bradshaw *et al.*, 1997);

2. Glyphosate has many unique properties, such as its mode of action, chemical structure, limited metabolism in plants, and lack of residual activity in soil, which make the development of resistance less likely;
3. Selection for glyphosate resistance using whole plant and cell/tissue culture techniques was unsuccessful, and would, therefore, be expected to occur rarely in nature under normal field conditions.

In 1996 in Australia, it was reported that a biotype of annual rye-grass (*Lolium rigidum*) was surviving application of label recommended rates of glyphosate (Pratley *et al*., 1996). To date, after examination of thousands of samples, only three locations have been confirmed as having the resistant population, indicating that the phenomenon is not widespread. A large body of biochemical and molecular biology experiments to determine the cause of observed weed control differences between Australian rye-grass biotypes resistant and susceptible to glyphosate indicate that the observed resistance is due to a combination of factors. Conclusions drawn to date are that the resistant biotype is easily controlled by conventional practices (tillages, other herbicides) and is caused by a complex inheritance pattern, unlikely to occur across a wide range of other species. Results of these studies have been presented (Pratley, 1999).

Additional reports of resistant ryegrass in northern California and South Africa are being investigated. Similar to the Australian locations, these fields are small and isolated. Again, the use of mowing and other herbicides have been very effective in controlling the ryegrass. Weed management recommendations are also in place and have successfully controlled the ryegrass. Research continues in an effort to better understand the resistance mechanism.

A population of *Elusine indica* (goosegrass) was reported to survive labeled rates of glyphosate in Malaysia. The fields from which these biotypes were collected had been treated an average of eight times per year with glyphosate for the past ten years. The glyphosate resistance observed in the field trials was confirmed in dose/pot greenhouse experiments. The analyses found that the resistant goosegrass has a modified EPSPS protein that is two-to-four-fold less sensitive to glyphosate than in more sensitive biotypes. Research is underway to investigate the resistance mechanism genetics and biology of the resistant biotype.

Most recently, observations of a resistant biotype of marestail (*Conyza canadensis*) were made in southern New Jersey, Delaware and western Tennessee. Marestail has a long history of being difficult to control with Roundup, so these isolated incidences were assumed to be weather related. An increase in reports prompted field visits and research was conducted to confirm that higher than labelled rates were necessary to control this biotype versus susceptible marestail plants. With this particular biotype, the most effective weed management plan to control this resistant population includes the use of herbicides with a mode of action other the inhibition of EPSPS.

Historically, the onset of resistance to glyphosate has been far less than with other products (HRAC *et al*., 2002). After 20 years of world wide use, confirmed resistance exists in only three plant species. Monsanto continues to aggressively monitor and investigate any such reports from customers. Weed management recommendations for Roundup Ready crops will continue to be

based on specific local needs and follow basic weed management principles. Weed management practices shall be structured to include Roundup alone, or in combination with other herbicides and/or cultural practices to deliver effective and economic weed control.

Environmental Assessment Conclusions

In summary, this assessment indicates that the environmental risks present with Roundup Ready corn are equivalent to or are not greater than those already present with conventional corn. Agronomic evaluations consisting of plant vigor, growth habit characteristics and general disease susceptibility have shown Roundup Ready corn to be unchanged compared to conventional corn. In addition, the introduced CP4 EPSPS proteins afford no significant potential for toxicity to wildlife or non-target organisms, and no detectable selective advantage outside of a field treated with glyphosate. Finally, data generated to support the registration of Roundup agricultural herbicides and almost 30 years of experience with glyphosate demonstrate that these herbicides will not cause unreasonable adverse effects to humans, mammals or other non-target organisms under normal use conditions. In addition, the data demonstrate that the use of these herbicides in corn is not expected to cause unreasonable adverse effects to the environment.

Summary

Weed control in corn is essential to protect against yield losses and to maintain grain and forage quality. In developed countries, this weed control is predominately achieved by chemical methods. The development of Roundup Ready corn enables the farmer to utilize Roundup agricultural herbicides for effective control of weeds during the corn-growing season and to take advantage of the herbicide's favorable environmental and safety characteristics. This in turn provides environmental benefits as well as significant value to the corn grower.

The introduction of Roundup Ready corn has reduced the number and cost of herbicide applications, and offers considerable environmental benefits due to its fit with conservation tillage systems. The introduced CP4 EPSPS and CP4 EPSPS L214P proteins are similar to other EPSPS proteins that are ubiquitous in nature. Detailed food, feed and environmental safety assessments confirm the safety of this product. The analyses included: 1) detailed molecular characterization of the introduced DNA; 2) safety assessments of the expressed CP4 EPSPS and CP4 EPSPS L214P proteins; 3) compositional analysis of corn grain and forage; 4) nutritional equivalence of corn grain in animal feeding studies; 5) a comparison of crop agronomic characteristics of NK603 corn to conventional corn hybrids; and 6) field observations to evaluate altered interactions with diseases and insect pests. These studies demonstrate that the CP4 EPSPS and CP4 EPSPS L214P proteins are not toxic to non-target organisms, including humans, animals and beneficial insects. Additionally, Roundup Ready corn plants containing corn event NK603 were shown to be as safe and nutritious as conventional corn varieties and to pose no greater environmental impact than conventional corn varieties.

Information and data contained within this document have been provided to regulatory authorities for review. Regulatory review continues as we update regulatory files and make

submissions to additional countries globally.

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Figure 1. Plasmid map of PV-ZMGT32 used to produce Roundup Ready corn event NK603.

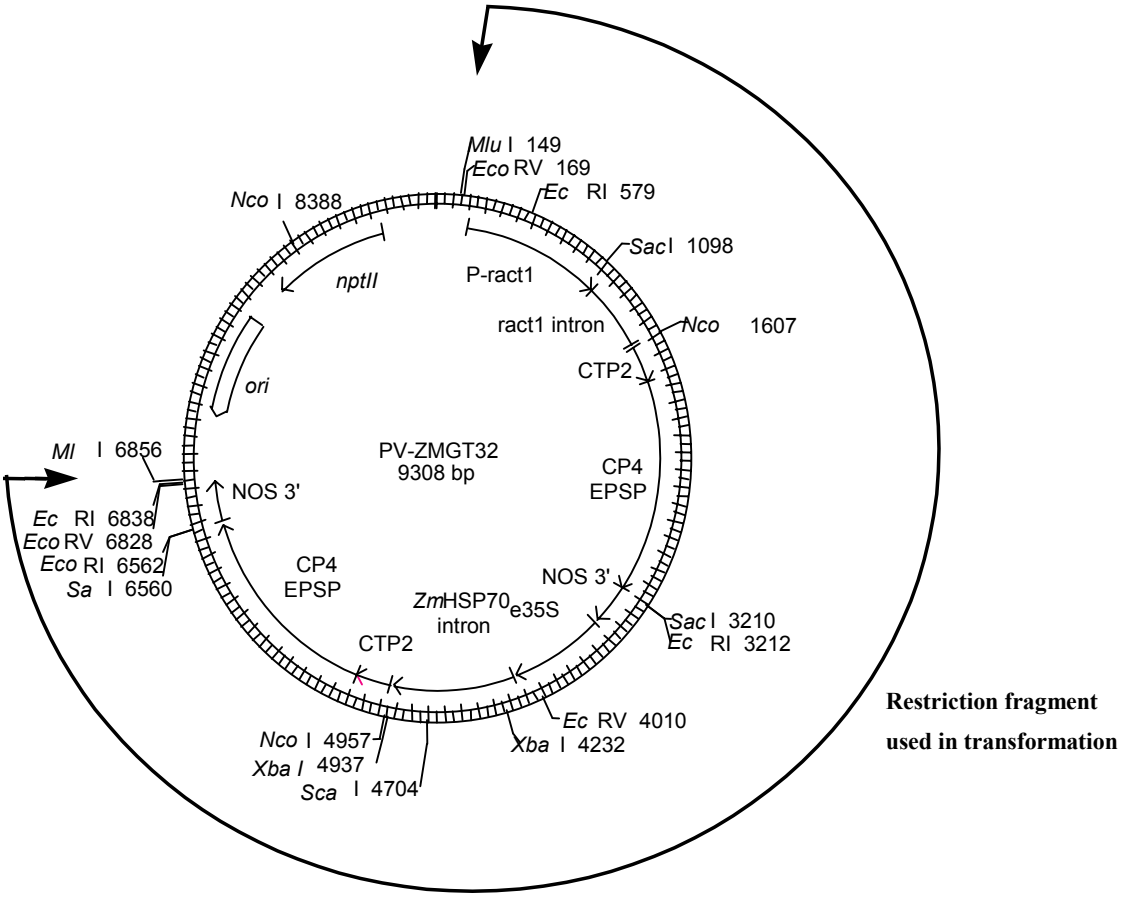

Table 1. Summary of CP4 EPSPS protein levels measured by ELISA in tissues of NK603 corn plants (µg/g fresh weight)

Parameter	Forage[a, c] (µg/g fw)	Grain[b, c] (µg/g fw)
Mean	25.6	10.9
Range	18.0 - 31.2	6.9 - 15.6
SD	3.8	2.6

SD = Standard Deviation.
[a]Limit of quantitation = 0.05 µg/g fw.
[b]Limit of quantitation = 0.09 µg/g fw.
[c]Values for all non-transgenic control samples were below the limit of quantitation of the assay.

Table 2. Fiber, Mineral and Proximate Composition of Grain from Corn Event NK603

Component[c]	1998[a]		1999[b]				
	NK603 Mean (Range)[h]	Control[d] Mean (Range)[h]	NK603 Mean (Range)[h]	Control[d] Mean (Range)[h]	Comm. Hybrids[e] Tolerance Interval[f] (Range)[h]	Literature (Range)	Historical[g] (Range)[h]
Protein	12.20 (10.30-14.77)	12.60 (11.02-14.84)	12.07 (10.23-13.92)	11.34 (10.13-13.05)	6.84, 14.57 (7.77-12.99)	(6.0-12.0)[k] (9.7-16.1)[l]	(9.0-13.6)
Total fat	3.61 (2.92-3.94)	3.67 (2.88-4.13)	4.16[j] (3.87-4.48)	3.60 (3.24-3.84)	1.55, 5.75 (2.57-4.95)	(3.1-5.7)[k] (2.9-6.1)[l]	(2.4-4.2)
Ash	1.45 (1.28-1.62)	1.49 (1.32-1.75)	1.38 (1.23-1.65)	1.34 (1.25-1.50)	0.77, 2.22 (1.02-1.94)	(1.1-3.9)[k]	(1.2-1.8)
ADF[i]	3.72 (3.14-5.17)	3.60 (2.79-4.28)	3.21 (2.63-3.87)	3.03 (2.30-3.68)	1.96, 4.71 (2.46-6.33)	(3.3-4.3)[k]	(3.1-5.3)
NDF[i]	10.06 (7.89-12.53)	10.00 (8.25-15.42)	10.08 (8.50-12.00)	10.57 (9.35-11.63)	7.26, 14.64 (8.45-14.75)	(8.3-11.9)[k]	(9.6-15.3)
Carbohydrates	82.76 (80.71-84.33)	82.29 (80.23-83.70)	82.39 (80.49-84.57)	83.73 (81.93-84.92)	79.38, 88.91 (82.18-88.14)	Not reported in this form	(81.7-86.3)
Moisture	11.13 (9.01-13.30)	11.78 (8.56-14.80)	7.62 (7.34-7.82)	7.81 (7.55-8.28)	7.06, 9.53 (7.43-9.94)	(7-23)[k]	(9.4-15.8)
Calcium	0.0047 (0.0037-0.0056)	0.0046 (0.0033-0.0058)	0.0053 (0.0050-0.0058)	0.0053 (0.0050-0.0058)	0.0028, 0.0082 (0.0039-0.0076)	(0.01-0.1)[k]	(0.003-0.006)
Copper	1.79 (1.19-2.37)	1.90 (1.50-2.33)	1.89 (1.77-1.99)	1.83 (1.69-1.97)	0.45, 3.16 (1.16-2.78)	(0.9-10)[k]	not available
Iron	22.71 (19.08-25.94)	22.95 (18.77-26.62)	22.73 (17.43-26.91)	21.81 (18.52-25.87)	10.60, 33.63 (15.42-29.34)	(1-100)[k]	not available

Table 2. Fiber, Mineral and Proximate Composition of Grain from Corn Event NK603 (continued)

Component[c]	1998[a]		1999[b]				
	NK603 Mean (Range)[h]	Control[d] Mean (Range)[h]	NK603 Mean (Range)[h]	Control[d] Mean (Range)[h]	Comm. Hybrids[e] Tolerance Interval[f] (Range)[h]	Literature (Range)	Historical[g] (Range)[h]
Magnesium	0.12 (0.11-0.13)	0.12 (0.11-0.13)	0.12 (0.096-0.13)	0.11 (0.10-0.12)	0.079, 0.16 (0.089-0.15)	(0.09-1.0)[k]	not available
Manganese	6.47 (4.64-9.63)	6.55 (4.96-8.83)	6.73 (5.18-7.90)	6.42 (5.63-7.32)	2.50, 12.03 (3.86-10.47)	(0.7-54)[k]	not available
Phosphorus	0.36 (0.32-0.39)	0.36 (0.32-0.39)	0.36 (0.31-0.39)	0.35 (0.32-0.37)	0.27, 0.42 (0.27-0.39)	(0.26-0.75)[k]	(0.288-0.363)
Potassium	0.36 (0.35-0.39)	0.36 (0.34-0.41)	0.36[j] (0.34-0.38)	0.38 (0.36-0.39)	0.31, 0.45 (0.32-0.45)	(0.32-0.72)[k]	not available
Zinc	28.35 (20.23-33.17)	28.72 (23.47-33.26)	23.78 (15.95-31.45)	23.21 (17.87-29.88)	9.89, 31.52 (13.51-27.98)	(12-30)[k]	not available

[a] Data from six non-replicated U.S. sites and two replicated U.S. sites; NK603 grain harvested from plants treated with Roundup Ultra®herbicide.
[b] Data from two replicated E.U. sites; NK603 grain harvested from plants treated with Roundup Ultra®herbicide.
[c] Percent dry weight of sample, except: moisture as percent fresh weight; copper, iron, manganese and zinc as mg/kg dry weight.
[d] Non-transgenic control hybrid.
[e] Commercial hybrids; local hybrids planted at each E.U. site.
[f] Tolerance interval is specified to contain 99% of the commercial line population, negative limits set to zero.
[g] Range for control hybrids planted in Monsanto Company field trials conducted in 1994 and 1995.
[h] Range denotes the lowest and highest individual value across sites for each hybrid.
[i] ADF = acid detergent fiber; NDF = neutral detergent fiber.
[j] Statistically significantly different from the control at the 5% level ($p<0.05$).
[k] Watson, 1987.
[l] Jugenheimer, 1976.

Table 3. Fiber, Mineral and Proximate Composition of Forage from Corn Event NK603

Component[c]	1998[a]		1999[b]			
	NK603 Mean (Range)[h]	Control[d] Mean (Range)[h]	NK603 Mean (Range)[h]	Control[d] Mean (Range)[h]	Comm. Hybrids[e] Tolerance Interval[f] (Range)[h]	Historical[g] (Range)
Protein	7.14 (5.57-8.98)	6.80 (5.49-8.69)	8.71 (6.37-10.79)	8.86 (7.03-10.96)	4.02, 12.46 (4.98-11.56)	(4.8-8.4)
Ash	3.81 (2.36-6.80)	4.02 (2.46-6.28)	4.38 (2.82-6.44)	4.44 (3.35-5.80)	0, 12.47 (2.43-9.64)	(2.9-5.1)
ADF[i]	25.72 (17.01-33.52)	24.84 (19.53-31.83)	23.53 (19.27-26.13)	22.07 (19.39-26.90)	9.80, 44.43 (17.54-38.31)	(21.4-29.2)
NDF[i]	42.09 (36.39-49.03)	42.45 (35.44-53.24)	37.34 (31.77-44.35)	37.75 (34.85-41.86)	20.77, 61.87 (27.93-54.75)	(39.9-46.6)
Total Fat	2.36 (0.69-3.64)	2.17 (0.61-3.42)	3.24 (2.06-4.49)	3.05 (2.09-4.02)	0.84, 4.80 (1.42-4.57)	(1.4-2.1)
Carbohydrates	86.71 (82.68-90.32)	87.11 (83.71-90.03)	83.67 (80.43-87.53)	83.65 (80.64-85.52)	75.55, 91.37 (76.50-87.29)	(84.6-89.1)
Moisture	67.02 (60.30-75.00)	66.24 (61.00-73.70)	67.53 (61.60-75.20)	66.30 (60.40-72.60)	45.40, 96.42 (56.50-80.40)	(68.7-73.5)

[a] Data from six non-replicated U.S. sites and two replicated U.S. sites; NK603 forage harvested from plants treated with Roundup Ultra®herbicide.
[b] Data from two replicated E.U. sites; NK603 forage harvested from plants treated with Roundup Ultra®herbicide.
[c] Percent dry weight of sample, except for moisture.
[d] Non-transgenic control hybrid.
[e] Commercial hybrids; local hybrids planted at each site.
[f] Tolerance interval is specified to contain 99% of the commercial line population, negative limits set to zero.
[g] Range for control hybrids planted in Monsanto Company field trials conducted in 1994 and 1995.
[h] Range denotes the lowest and highest individual value across sites for each hybrid.
[i] ADF = acid detergent fiber; NDF = neutral detergent fiber.

Table 4. Amino Acid Composition of Corn Grain from Corn Event NK603

Amino acid[a]	1998[b]		1999[c]				
	NK603 Mean (Range)[i]	Control[d] Mean (Range)[i]	NK603 Mean (Range)[i]	Control[d] Mean (Range)[i]	Comm. Hyrids[e] Tolerance Interval[f] (Range)[i]	Literature[g] (Range)	Historical[h] (Range)[i]
Alanine	7.93 (7.78-8.22)	7.89 (7.65-8.17)	8.04[j] (7.87-8.18)	7.95 (7.88-8.05)	7.20, 8.35 (7.38-8.13)	(6.4-9.9)	(7.2-8.8)
Arginine	4.16 (3.79-4.49)	4.24 (3.90-4.63)	4.00[j] (3.74-4.27)	4.27 (4.09-4.36)	3.45, 5.03 (3.77-4.98)	(2.9-5.9)	(3.5-5.0)
Aspartic Acid	6.45 (6.29-6.62)	6.40 (6.18-6.56)	6.45 (6.27-6.96)	6.28 (6.18-6.37)	5.53, 7.61 (6.02-7.51)	(5.8-7.2)	(6.3-7.5)
Cysteine/Cystine	2.00 (1.69-2.27)	2.00 (1.63-2.22)	1.82 (1.66-1.98)	1.92 (1.61-2.09)	1.56, 2.43 (1.68-2.51)	(1.2-1.6)	(1.8-2.7)
Glutamic Acid	19.84 (19.16-20.47)	19.81 (19.19-20.41)	19.93[j] (18.98-20.62)	19.40 (18.69-19.92)	18.03, 20.76 (18.38-20.08)	(12.4-19.6)	(18.6-22.8)
Glycine	3.49 (3.22-3.74)	3.51 (3.22-3.86)	3.44 (3.23-3.64)	3.60 (3.44-3.77)	3.06, 4.15 (3.27-4.01)	(2.6-4.7)	(3.2-4.2)
Histidine	2.72 (2.45-2.81)	2.74 (2.56-2.88)	2.65[j] (2.56-2.74)	2.77 (2.69-2.85)	2.34, 3.36 (2.58-3.15)	(2.0-2.8)	(2.8-3.4)
Isoleucine	3.87 (3.59-4.06)	3.80 (3.65-3.93)	3.77 (3.54-3.97)	3.76 (3.61-3.85)	3.35, 3.97 (3.34-3.85)	(2.6-4.0)	(3.2-4.3)
Leucine	14.20 (13.63-14.79)	14.07 (13.59-14.60)	14.02 (13.38-14.71)	13.69 (13.27-13.96)	11.73, 14.76 (12.18-14.34)	(7.8-15.2)	(12.0-15.8)

Table 4. Amino Acid Composition of Corn Grain from Corn Event NK603 (continued)

Amino acid[a]	1998[b]		1999[c]				
	NK603 Mean (Range)[i]	Control[d] Mean (Range)[i]	NK603 Mean (Range)[i]	Control[d] Mean (Range)[i]	Comm. Hyrids[e] Tolerance Interval[f] (Range)[i]	Literature[g] (Range)	Historical[h] (Range)[i]
Lysine	2.69 (2.42-2.96)	2.67 (2.35-3.00)	2.71[j] (2.37-3.03)	2.83 (2.56-3.20)	2.22, 3.68 (2.58-3.67)	(2.0-3.8)	(2.6-3.5)
Methionine	1.94 (1.76-2.16)	2.03 (1.74-2.21)	1.77[j] (1.66-1.85)	1.89 (1.67-2.06)	1.39, 2.49 (1.49-2.32)	(1.0-2.1)	(1.3-2.6)
Phenylalanine	5.32 (5.18-5.52)	5.24 (5.09-5.36)	5.28 (5.13-5.46)	5.25 (5.20-5.29)	4.59, 5.61 (4.85-5.54)	(2.9-5.7)	(4.9-6.1)
Proline	8.88 (8.44-9.10)	8.96 (8.59-9.26)	9.33 (8.89-9.71)	9.16 (8.83-9.31)	8.61, 10.09 (8.74-9.91)	(6.6-10.3)	(8.7-10.1)
Serine	4.87 (4.72-5.09)	4.86 (4.68-4.99)	4.84 (4.47-5.17)	4.90 (4.82-5.09)	4.36, 5.19 (4.41-5.22)	(4.2-5.5)	(4.9-6.0)
Threonine	3.37 (3.26-3.46)	3.33 (3.19-3.50)	3.31 (3.14-3.57)	3.29 (3.15-3.50)	3.14, 3.69 (3.24-3.66)	(2.9-3.9)	(3.3-4.2)
Tryptophan	0.53 (0.44-0.58)	0.54 (0.48-0.60)	0.58 (0.49-0.64)	0.62 (0.57-0.69)	0.45, 0.76 (0.49-0.79)	(0.5-1.2)	(0.4-1.0)
Tyrosine	3.02 (2.36-3.73)	3.25 (2.43-3.64)	3.24 (2.11-3.65)	3.52 (2.69-3.69)	3.00, 4.03 (2.32-3.90)	(2.9-4.7)	(3.7-4.3)

Table 4. Amino Acid Composition of Corn Grain from Corn Event NK603 (continued)

Amino acid[a]	1998[b]		1999[c]				
	NK603 Mean (Range)[i]	Control[d] Mean (Range)[i]	NK603 Mean (Range)[i]	Control[d] Mean (Range)[i]	Comm. Hyrids[e] Tolerance Interval[f] (Range)[i]	Literature[g] (Range)	Historical[h] (Range)[i]
Valine	4.74 (4.59-4.85)	4.71 (4.62-4.94)	4.81 (4.55-5.00)	4.90 (4.74-5.04)	4.64, 5.38 (4.65-5.29)	(2.1-5.2)	(4.2-5.3)

[a] Values expressed as percent of total amino acids for statistical comparisons.
[b] Data from six non-replicated U.S. sites and two replicated U.S. sites; NK603 grain harvested from plants treated with Roundup Ultra®herbicide.
[c] Data from two replicated E.U. sites; NK603 grain harvested from plants treated with Roundup Ultra®herbicide.
[d] Non-transgenic control line.
[e] Commercial lines; local hybrids planted at each E.U. site.
[f] Tolerance interval is specified to contain 99% of the commercial line population, negative limits set to zero.
[g] Watson, 1982. Values are percent of total protein [10.1% total protein (N x 6.25)].
[h] Range for control hybrids planted in Monsanto Company field trials conducted between 1993 and 1995; values are percent of total protein.
[i] Range denotes the lowest and highest individual value across sites.
[j] Value statistically significantly different than the control at the 5% level ($p<0.05$).

Table 5. Fatty Acid Composition of Corn Grain from Corn Event NK603

Fatty acid[a]	1998[b]		1999[c]				
	NK603 Mean (Range)[i]	Control[d] Mean (Range)[i]	NK603 Mean (Range)[i]	Control[d] Mean (Range)[i]	Comm. Hybrids[e] Tolerance Interval[f] (Range)[i]	Literature[g] (Range)	Historical[h] (Range)[i]
Arachidic (20:0)	0.36 (0.34-0.39)	0.37 (0.33-0.40)	0.36[j] (0.34-0.39)	0.35 (0.33-0.37)	0.17, 0.64 (0.31-0.74)	(0.1-2)	(0.3-0.5)
Behenic (22:0)	0.16 (0.14-0.19)	0.16 (0.14-0.19)	0.16 (0.12-0.20)	0.18 (0.15-0.19)	0.093, 0.24 (0.073-0.22)	(not reported)	(0.1-0.3)
Eicosenoic (20:1)	0.29 (0.28-0.32)	0.30 (0.27-0.34)	0.30 (0.28-0.34)	0.29 (0.28-0.31)	0.21, 0.42 (0.26-0.40)	(not reported)	(0.2-0.3)
Linoleic (18:2)	64.62 (63.79-65.80)	64.26 (63.07-65.65)	63.73 (61.94-65.25)	63.15 (61.63-64.04)	44.59, 73.50 (49.72-65.98)	(35-70)	(55.9-66.1)
Linolenic (18:3)	1.11 (1.07-1.17)	1.11 (1.07-1.20)	1.02 (0.97-1.05)	1.09 (1.05-1.12)	0.54, 1.72 (0.71-1.50)	(0.8-2)	(0.8-1.1)
Oleic (18:1)	22.40[j] (21.37-23.12)	23.08 (22.15-24.14)	23.80 (22.82-24.95)	24.20 (23.52-25.56)	12.65, 39.86 (20.21-34.64)	(20-46)	(20.6-27.5)
Palmitic (16:0)	9.13[j] (8.67-9.57)	8.89 (8.41-9.44)	8.90 (8.47-9.36)	9.00 (8.89-9.13)	7.35, 14.72 (9.12-12.62)	(7-19)	(9.9-12.0)

Table 5. Fatty Acid Composition of Corn Grain from Corn Event NK603 (continued)

Fatty acid[a]	1998[b]		1999[c]				
	NK603 Mean (Range)[i]	Control[d] Mean (Range)[i]	NK603 Mean (Range)[i]	Control[d] Mean (Range)[i]	Comm. Hybrids[e] Tolerance Interval[f] (Range)[i]	Literature[g] (Range)	Historical[h] (Range)[i]
Stearic (18:0)	1.92[j] (1.80-2.06)	1.83 (1.67-1.98)	1.73 (1.59-1.88)	1.74 (1.67-1.81)	1.02, 2.27 (1.19-2.02)	(1-3)	(1.4-2.2)

[a] Value of fatty acids expressed as % of total fatty acid. The method included the analysis of the following fatty acids which were not detected in the majority of samples analyzed: caprylic acid (8:0), capric acid (10:0), lauric acid (12:0), myristic acid (14:0), myristoleic acid (14:1), pentadecanoic acid (15:0), pentadecenoic acid (15:1), palmitoleic acid (16:1), heptadecanoic acid (17:0), heptadecenoic acid (17:1), gamma linolenic (18:3), eicosadienoic acid (20:2), eicosatrienoic acid (20:3), and arachidonic acid (20:4).

[b] Data from six non-replicated U.S. sites and two replicated U.S. sites; NK603 grain harvested from plants treated with Roundup Ultra®herbicide.

[c] Data from two replicated E.U. sites; NK603 grain harvested from plants treated with Roundup Ultra®herbicide.

[d] Non-transgenic control hybrid.

[e] Commercial hybrids; local hybrids planted at each E.U. site.

[f] Tolerance interval is specified to contain 99% of the commercial line population, negative limits set to zero.

[g] Watson, 1982. Values expressed as % of total fat except for palmitic acid (16:1) which is expressed as % of triglyceride fatty acids.

[h] Range for control lines planted in Monsanto Company field trials conducted between 1993 and 1995; values are expressed as % of total fatty acids.

[i] Range denotes the lowest and highest individual value across sites.

[j] Statistically significantly different from the control at the 5% level ($p<0.05$).

Table 6. Phytic Acid, Trypsin Inhibitor, Vitamin E and Secondary Metabolite Content of Corn Grain from Corn Event NK603

Component	1998[a]		1999[b]				
	NK603 Mean (Range)[h]	Control[c] Mean (Range)[h]	NK603 Mean (Range)[h]	Control[c] Mean (Range)[h]	Comm. Hybrids[d] Tolerance Interval[e] (Range)[h]	Literature[f] (Range)	Historical[g] (Range)
Phytic Acid (% dw)	0.97 (0.70-1.06)	1.00 (0.81-1.21)	0.79 (0.51-0.89)	0.70 (0.55-0.77)	0.32, 1.18 (0.48-1.12)	to 0.9%	not available
Trypsin Inhibitor (TIU/mg dw)	3.16 (2.34-5.08)	2.67 (1.39-5.14)	1.56 (0.54-2.57)	1.15 (0.54-2.38)	0, 3.63 (0.54-4.13)	not available	not available
Vitamin E (mg/g dw)	0.0088 (0.0070-0.010)	0.0090 (0.0064-0.011)	0.0062 (0.0046-0.0080)	0.0070 (0.0050-0.014)	0, 0.021 (0.0027-0.015)	(0.017-0.047)	(0.008-0.015)[h]
Ferulic Acid (% dw)	0.20 (0.15-0.25)	0.20 (0.17-0.23)	not available	not available	not available	not available	(0.17-0.27)[i]
p-Coumaric Acid (%dw)	0.016 (0.012-0.022)	0.015 (0.012-0.020)	not available	not available	not available	not available	(0.011-0.030)[i]
Raffinose (% dw)	0.13 (0.098-0.20)	0.13 (0.082-0.21)	not available	not available	not available	not available	(0.053-0.16)[i]

[a] Data from six non-replicated U.S. sites and two replicated U.S. sites; NK603 grain harvested from plants treated with Roundup Ultra®herbicide.
[b] Data from two replicated E.U. sites; NK603 grain harvested from plants treated with Roundup Ultra®herbicide.
[c] Non-transgenic control hybrid.
[d] Commercial hybrids; local hybrids planted at each E.U. site.
[e] Tolerance interval is specified to contain 99% of the commercial line population, negative limits set to zero.
[f] Watson, 1987.
[g] Range for control hybrids planted in Monsanto Company field trials conducted between 1993 and 1995.
[h] Range denotes the lowest and highest individual value across sites for each hybrid.
[i] Range for thirteen commercial hybrids planted in Monsanto Company field trials or purchased from growers in 1998.

Table 7. Performance of broilers, carcass yield and protein and fat composition of breast and thighs (mean values of males and females) and comparison of transgenic corn event NK603 with control and reference lines.

Corn Line	NK603	B73HT x LH82	RX826	LH235 x LH185	DK493	MON847	RX770	Treatments (T) SSD[1]	LSD[2] 5.0%	Historical Range[3,5]	Literature Range[4]
Performance											
Live weight (g/bird) day 0	38.183	38.417	38.500	38.100	38.383	38.333	38.250	NS	0.7970	NA	NA
Live weight (kg/pen) day 0	0.46	0.46	0.46	0.46	0.46	0.46	0.46	NS	0.009	NA	NA
Live weight (kg/bird) day 42	2.301	2.310	2.337	2.346	2.327	2.318	2.253	NS	0.0688	1.891-2.190	1.79-2.43[a-f]
Live weight (kg/pen) day 42	22.770	22.850	23.370	22.720	22.760	22.480	22.530	NS	1.1087	14.73-21.90	NA
Feed intake (kg/bird)	3.547	3.586	3.694	3.706	3.689	3.667	3.543	NS	0.1318	NA	NA
Feed intake (kg/pen)	35.090	35.470	36.940	35.870	36.040	35.570	35.430	NS	1.4846	25.44-34.04	NA
Feed efficiency (kg/kg)	1.543	1.555	1.585	1.581	1.587	1.587	1.574	NS	0.0320	1.555-1.782	1.60-2.07[a,b,c,d]
Adjusted Feed Efficiency (kg/kg)	1.528[c]	1.546[bc]	1.573[a]	1.549[bc]	1.556[ab]	1.563[ab]	1.563[ab]	*	0.0240	1.545-1.724	NA
Carcass Yield											
Live weight (kg)	2.246	2.225	2.299	2.287	2.263	2.254	2.195	NS	0.0658	NA	NA
Chill weight (kg)	1.592	1.580	1.637	1.622	1.605	1.598	1.556	NS	0.0515	NA	NA
Chill weight (% of live weight)	70.90	71.00	71.20	70.90	70.90	70.90	70.80	NS	0.4600	NA	67.1-76.0[a,c,d,e]
Fat pad weight (kg)	0.034[b]	0.037[a]	0.036[ab]	0.039[a]	0.039[a]	0.037[a]	0.037[a]	*	0.0028	0.0337-0.0441	0.0242-0.0632[a-f]
Fat pad weight (% of live weight)	1.5[c]	1.7[ab]	1.6[bc]	1.7[a]	1.7[a]	1.7[ab]	1.7[ab]	**	0.1100	1.80-2.18	1.14-3.60[a-f]
Breast meat weight (kg)	0.407[abcd]	0.394[d]	0.423[a]	0.415[ab]	0.413[abc]	0.404[bcd]	0.394[cd]	*	0.0183	NA	0.225-0.551[a,b,d,e]
Breast meat weight (% of chill wt.)	25.50	24.90	25.80	25.60	25.70	25.30	25.30	NS	0.5400	NA	11.19-32.62[a,d,e]
Thighs weight (kg)	0.279	0.275	0.282	0.277	0.274	0.276	0.268	NS	0.0101	NA	0.258-0.318[e,f]
Thighs weight (% of chill wt.)	17.50	17.40	17.20	17.10	17.10	17.30	17.20	NS	0.2900	NA	12.80-20.65[e,f]
Drums weight (kg)	0.227	0.224	0.231	0.227	0.225	0.227	0.223	NS	0.0074	NA	0.213[f]
Drums weight (% of chill wt.)	14.30	14.20	14.10	14.00	14.00	14.20	14.30	NS	0.2500	NA	10.50[f]
Wings weight (kg)	0.186	0.185	0.191	0.188	0.187	0.185	0.182	NS	0.0055	NA	0.170[f]
Wing weight (% of chill wt.)	11.70	11.80	11.70	11.60	11.70	11.60	11.70	NS	0.1400	NA	8.40[f]

Table 7. (con't.) Performance of broilers, carcass yield and protein and fat composition of breast and thighs (mean values of males and females) and comparison of transgenic corn event NK603 with control and reference lines (continued)

Corn Line	NK603	B73HT x LH82	RX826	LH235 x LH185	DK493	MON847	RX770	Treatments (T) SSD[1]	LSD[2] 5.0%	Historical Range[3,5]	Literature Range[4]
Breast Meat Analysis											
Moisture (%)	74.741	74.879	74.716	74.726	74.774	74.993	74.439	NS	0.4669	NA	72.7-74.3[g]
Protein (%, as is basis)	24.111	23.712	24.235	24.346	24.157	24.008	24.019	NS	0.5355	NA	22.9-24.3[g]
Fat (%, as is basis)	0.867	0.931	0.810	1.035	0.809	1.036	0.798	NS	0.1987	NA	0.770-1.80[g]
Thigh Meat Analysis											
Moisture (%)	75.894[bc]	75.752[c]	76.360[ab]	76.606[a]	76.293[ab]	76.804[a]	76.039[bc]	**	0.5203	NA	70.0-72.4[g]
Protein (%, as is basis)	21.061	20.502	21.161	21.133	21.025	20.659	21.339	NS	0.5538	NA	17.7-19.2[g]
Fat (%, as is basis)	2.455	2.311	1.966	1.847	2.139	1.833	2.153	NS	0.5661	NA	7.50-11.6[g]

[1] SSD, statistical significance of differences: NS, not significant; *, P<0.05; **, P<.01; Individual treatment means with the same superscript letter in the same row are not statistically different (P>0.05).
[2] LSD, least significant difference between two means (P<0.05).
[3] 38-42 day Monsanto studies numbered XX-97-252 (Ross x Arbor Acres) and XX-98-081 (Ross x Ross).
[4] *a*) Smith, *et al.,* 1998 (Ross x Ross); *b*) Lei and Van Beek, 1997 (Ross x Ross); *c*) Farran, *et al*., 2000 (Ross); *d*) Esteve-Garcia and Llaurado, 1997 (Ross); *e*) Kidd and Kerr, 1997 (Ross x Ross); *f*) Peak, *et al*., 2000 (Ross x Ross, Cobb x Cobb, and Ross x Cobb); and *g*) Grey, *et al*., 1983 (Ross).
[5]NA, not available.