SUMMARY REPORT

RISK ASSESSMENT OF GENETICALLY MODIFIED ROUNDUP READY® CORN 2 NK603 TO ENVIRONMENT AND BIODIVERSITY

VIETNAM

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I. General Information

1. Name of Applicant: Dekalb Vietnam Company Limited

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2. Name of GM plants

Common name: Corn or Maize
Scientific name: *Zea mays L.*
Trade name: Genuity Roundup Ready® 2 corn
Gene transfer event: The herbicide tolerant corn, NK603
Introduced trait related to the transformed gene: Herbicide tolerance
Purpose of use: in country cultivation
The only identified code (if yes): MON-ØØ6Ø3-6

3. Brief Description of Phenotypic Effect(s) of the Transgene:

NK603 contains two *cp4 epsps* genes that encode two near-identical glyphosate-tolerant EPSPS proteins (CP4 EPSPS) that confer tolerance to glyphosate, the active ingredient in Roundup agricultural herbicides.

4. Method of Transformation used:

Agrobacterium-mediated transformation

II. Executive Summary and Overall Conclusions

Monsanto Company, USA has developed, through the use of recombinant DNA techniques, corn (*Zea mays* L.) plants which are commercially tolerant to glyphosate, the active ingredient in Roundup® agricultural herbicides. The tissues of these plants produce a glyphosate-tolerant EPSPS from *Agrobacterium* sp. strain CP4 (CP4 EPSPS). This particular protein has also been used in other Roundup Ready crops, soybean, canola, sugar beet and cotton and these crops have been planted in many countries around the world for over 15 years. In nontransgenic plants, glyphosate binds to the plant EPSPS enzyme and blocks the biosynthesis of aromatic amino acids, thereby preventing production of these essential compounds. The CP4 EPSPS enzyme, however, has a reduced affinity for glyphosate when compared to the native corn EPSPS enzyme, which is inhibited by glyphosate (1). As a result, corn plants expressing the CP4 EPSPS protein, when treated with glyphosate, are unaffected, as the continued action of the tolerant enzyme provides the plant's need for aromatic amino acids.

Roundup Ready® corn 2 (NK603) contains two *cp4 epsps* genes that encode two near-identical glyphosate-tolerant EPSPS proteins (CP4 EPSPS) that confer tolerance to glyphosate, the active ingredient in Roundup agricultural herbicides. The *epsps* (5-enolpyruvylshikimate-3-phosphate synthase) coding sequences from *Agrobacterium* sp. strain CP4 were transferred into the genome of conventional corn using a particle acceleration transformation system.

In Vietnam, corn is grown over an area of approximately one million hectares with total production of about 4.43 million tones. Worldwide maize grain production averaged over 780 million metric tons (MMT) per year from 2006 to 2010 (2). Among the corn cultivation countries, USA has the largest area (22%) followed by China (18%), Brazil (9%), and India (5.2%). The USA also leads in production of corn grain (42%) followed by China (19%), with India at the sixth position with a share of 2.4%.

Weed control is essential in cornfields, as weeds compete with the crop for sunlight, water and nutrients. Failure to control weeds results in decreased yields and reduced crop quality. The introduction of NK603 in Vietnam is expected to provide significant benefits to farmers by allowing over the top use of Roundup herbicide for the control of economically important weeds without damage to the corn plant. In addition the use of glyphosate in conjunction with NK603 will allow the grower to utilize reduced tillage techniques, which provide significant environmental benefits, such as reduced soil erosion, reduced use of fossil fuels, and improved soil quality.

NK603 was produced by biolistic transformation of the inbred corn line LH82xB73 using a linear DNA fragment, PV-ZMGT32L which contains two *cp4 epsps* gene cassettes in tandem. The DNA segment used for transformation was isolated as a single band following agarose gel electrophoresis of restriction enzyme digested PV-ZMGT32 plasmid DNA. The purified DNA fragment did not contain antibiotic resistance marker genes, bacterial origin of replication sequences, or any plasmid derived sequences. Transformed plant cells were selected and regenerated in tissue culture in the presence of glyphosate.

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The data and information presented in this application demonstrate that NK603 corn is not likely to pose an increased plant pest potential or to have an adverse environmental impact compared to conventional corn. This conclusion is based on several lines of evidence. The first is the detailed molecular characterization of the insert DNA. Results confirm the insertion of a single functional copy of the *cp4 epsps* expression cassettes at a single locus within the genome. The second is a thorough assessment of the allergenicity and toxicity potential of the CP4 EPSPS and CP4 EPSPS L214P proteins based on extensive studies. The results demonstrate the safety of the CP4 EPSPS and CP4 EPSPS L214P proteins due to the lack of allergenic potential and the lack of acute toxicity when ingested, and their similarity to CP4 EPSPS proteins that have a history of safe use. The third is the estimation of protein levels and dietary safety assessment based on anticipated exposure and the results acute toxicology tests. Results show that there are no meaningful risks to human or animal health from dietary exposure to the CP4 EPSPS proteins based on the large margins of exposure (MOEs) obtained. Most importantly, the compositional and nutritional assessment of NK603 grain and forage confirms that NK603 is compositionally equivalent to and as safe as conventional corn. Moreover, extensive evaluation of the NK603 corn phenotypic and agronomic characteristics and ecological interactions in many environments in the world including two years of confined field trials conducted in Vietnam, demonstrates that NK603 is not likely to have an increased plant pest potential compared to the conventional corn. Finally, an assessment on the potential impact on non-target organisms (NTO) and endangered species concludes that NK603 is unlikely to have adverse effects on these organisms under the proposed conditions of use

Molecular characterization of NK603 by Southern blot analyses was conducted to determine: 1) the number of inserts and copies in the genome, 2) intactness of the genetic elements within the insert, 3) absence of the T-DNA II encoding the selectable marker, 4) absence of backbone sequences, and 5) stability of the inserted DNA across multiple generations. The integrated DNA in NK603 was characterized by Southern blot analyses. The data indicate that NK603 contain one copy of the insert at a single integration locus and all expression elements are present. These data also demonstrated that NK603 does not contain detectable backbone or selectable marker sequence from plasmid PV-ZMGT32L. Finally, the complete DNA sequence of the insert and adjacent genomic DNA sequence in NK603 were determined, which confirmed the reported organization of the elements within the insert and identified the 5' and 3' insert-to-genomic DNA junctions.

The amount of protein required for the toxicity studies can not be generated directly from the plant because of the relatively low levels of protein actually expressed in the plant. Thus, it is standard practice to produce the protein from a heterologous source like bacteria and then establish the equivalence to the plant produced protein prior to using it for toxicity testing. The NK603- and bacterially-produced CP4 EPSPS proteins were shown to be equivalent based on several criteria, including Western blot assay and a phosphate release functional assay. The CP4 EPSPS and CP4 EPSPS L214P proteins were also shown to be physicochemically and functionally equivalent based on structural and modeling criteria using the known X-ray crystal structure of CP4 EPSPS and the phosphate release functional assay.

The assessment of potential allergenicity and toxicity showed there was a reasonable certainty of no harm to mammals from exposure to the CP4 EPSPS and CP4 EPSPS P214L proteins. These assessments were based on; a) an evaluation of potential allergenicity based on the source of the protein, structural similarities to known allergens, *in vitro* digestibility in simulated digestive

fluids, and expected dietary exposure and, b) an evaluation of potential toxicity based on history of safe use of EPSPS proteins, lack of similarity to known toxins or biologically active proteins and an evaluation of acute toxicity to mammals.

CP4 EPSPS is functionally similar to the EPSPS proteins typically present in foods and feeds derived from plant and microbial sources, based on the reaction catalyzed. As mentioned previously, *Agrobacterium* sp. strain CP4, the donor organism for these two CP4 EPSPS proteins, has been used commercially in the U.S., is not known for human or animal pathogenicity, is not commonly allergenic and do not warrant analytical or toxicological tests. Furthermore, according to FAO/WHO (3), there is no known population of individuals sensitized to bacterial proteins. Potential allergenicity was assessed based on knowledge of the source organism of the protein, an assessment of structural similarities to known allergens, *in vitro* digestibility in simulated digestive fluids, and abundance of the proteins in corn grain. The CP4 EPSPS and CP4 EPSPS L214P proteins together are present at approximately 0.01 % of the total protein found in the grain of NK603. The low levels of the CP4 EPSPS proteins in NK603, combined with the rapid digestibility of these proteins in the in vitro digestibility test, establishes an extremely low probability of the CP4 EPSPS proteins that they are likely to be allergens. Results of extensive bioinformatics assessments using FASTA sequence alignment and eight-amino acid sliding window searches showed that the CP4 EPSPS and CP4 EPSPS L214P proteins do not share any amino acid sequence similarities with known allergens, gliadins, glutenins, or protein toxins that have adverse effects to mammals. Mice acute oral toxicity studies demonstrate that no adverse effects were observed when CP4 EPSPS was administered at levels up to 572 mg/kg of body weight. The results of the searches revealed no biologically relevant sequence similarities between CP4 EPSPS and known toxins. Similarly, no adverse effects were observed when CP4 EPSPS L214P was administered to mice at levels up to 817 mg/kg of body weight.

The expression levels of CP4 EPSPS proteins were determined by validated enzyme-linked immunosorbent assays (ELISA) in corn tissues of NK603 collected from plants grown at six non-replicated and two replicated field sites during the 1998 United States growing season. ELISA analyses were conducted on forage and grain. Protein levels for each tissue type were calculated on a microgram (µg) per gram (g) fresh weight (fwt) basis. Moisture content was then measured for each tissue type from each site and protein levels were converted and reported on a dry weight (dwt) basis.

Results showed that mean CP4 EPSPS protein levels in NK603 forage were comparable for the non-replicated sites (25.5 µg/g fw) and replicated sites (25.9 µg/g fw). CP4 EPSPS protein levels in control forage were, as expected, below the Limit of Quantitation of the assay (<0.05 µg/g fw). Mean CP4 EPSPS protein levels in NK603 grain were comparable for the non-replicated sites (11.0 µg/g fw) and replicated sites (10.6 µg/g fw). CP4 EPSPS protein levels in control grain were, as expected, below the Limit of Quantitation of the assay (<0.09 µg/g fw). Therefore, it is concluded that the CP4 EPSPS protein introduced into NK603 is expressed at approximately the same levels within site or across geographically dispersed sites. This low level of CP4 EPSPS protein expression in NK603 is sufficient to confer tolerance to glyphosate, the active ingredient in Roundup herbicide.

Compositional assessment of the grain and forage from NK603 demonstrated that it is nutritionally and biologically equivalent to its conventional counterpart. Compositional data on key nutrients, anti-nutrients and other components were collected for the forage and grain from NK603 and conventional control corn, grown at field sites in the U.S. in 1998 and in the European Union in 1999. Compositional analyses were performed to measure proximates, fiber, amino acids, fatty acids, vitamin E, nine minerals, phytic acid, trypsin inhibitor, and secondary metabolites in grain as well as proximates and fiber in forage.

The combined-site values for all of the biochemical components assessed for NK603 were similar to those of the nontransgenic control or were within the published range observed for nontransgenic commercial corn hybrids. In addition, the compositional profile of NK603 was compared with that of traditional corn hybrids grown in Europe by calculating a 99% tolerance interval to describe compositional variability in the population of traditional corn varieties in the marketplace. These comparisons, together with the history of the safe use of corn as a common component of animal feed and human food, support the conclusion that NK603 is compositionally equivalent to, and as safe and nutritious as, conventional corn hybrids grown commercially today.

The phenotypic, agronomic, and ecological interaction assessment for NK603 corn has been conducted in field trials in many locations in several countries including the United States. An important element in assessing plant pest potential and environmental impact was to compare NK603 to the conventional counterpart when grown side by side. The assessment is based initially on the concept of familiarity, which USDA recognizes plays an important role in assessments. Familiarity is based on the fact that the biotechnology-derived plant is developed from a conventional plant variety whose biological properties and plant pest potential are known to experts. Familiarity considers the biology of the crop, the introduced trait, the receiving environment and the interaction among these factors, and provides a basis for comparative risk assessment between a biotechnology-derived plant and its conventional counterpart. The assessments are based on a combination of laboratory experiments and field studies conducted by scientists who are familiar with the production and evaluation of corn. In each of these studies, NK603 was compared to an appropriate conventional corn hybrid which has a genetic background similar to NK603 but does not possess the herbicide tolerance trait. In addition, multiple commercial corn hybrids were also employed to provide a range of values that are common to the commercial corn hybrids for each measured characteristic. These assessments included seed germination parameters, pollen characteristics, plant growth and development characteristics, and numerous observations for each of the plant-disease and plant responses to abiotic stressor interactions.

Based on the environmental consequences of the introduction of corn line NK603 that were evaluated, there is no reason to believe that line NK603 would have a significant adverse impact on organisms beneficial to plants or to non-target organisms including threatened or endangered species. The environmental consequences of pollen transfer by wind pollination from NK603 to other corn is considered to be negligible due to the safety of the CP4 EPSPS protein and the lack of any selective advantage conferred on the recipient corn plant. The agronomic consequences of volunteer corn plants would be minimal as the plants are easily controlled by mechanical means, or by one of a number of other herbicides currently registered for corn. Also, there are no significant populations of sexually compatible related species of corn (e.g. teosinte; *Zea mays* ssp. *mexicana*) in Vietnam.

Also, an extensive set of data collected over two years (2010 and 2011) from confined field trials in Vietnam were used to assess whether the introduced traits altered the plant pest, weediness potential and effects with NK603 alone and in combination with the insect protected trait, MON 89034, when compared to conventional corn. Phenotypic, agronomic, and susceptibility to diseases of those glyphosate tolerance traits were evaluated and compared to their conventional counterpart. These assessments included plant growth and development characteristics; diseases and pests damage and efficacy on grain yield. Results from the phenotypic, agronomic, and non-target organisms assessment indicate that NK603 and MON 89034 × NK603 do not possess weedy characteristics, increased susceptibility or tolerance to specific diseases or characteristics that would confer a plant pest risk or significant environmental impact compared to conventional corn.

The efficacy of NK603 and MON 89034 x NK603 was evaluated to determine the effects of Roundup Herbicide on NK603 and MON 89034 x NK603. In an assessment in an individual location, NK603 and MON 89034 x NK603 provided excellent weed control in the corn field and crop safety. Percent weed control were statistically analyzed only within individual observation showed Roundup herbicide at all application rates provided an effective control in controlling both grasses as well as broad-leaf weeds compared to Atrazine treated control (C919 T1) and unweeded control (C919 T4). No chlorosis, necrosis or herbicide-induced damage was observed on NK603, MON 89034 × NK603 maize plants at any application rate of Roundup herbicide. Furthermore, the high level of weed control of NK603 and MON 89034 x NK603 contributed to an improved agronomic performance compared to the counterpart control C919.

Overall all data and information in this submission demonstrates that NK603 does not represent a unique plant pest risk or to have an adverse environmental impact compared to conventional corn. The successful adoption of NK603 is expected to increase economic, environmental and health benefits due to the protection of corn yields, usage of safer herbicide (limited metabolism in plant, lack of residual activity in soil) for weed management and increase adoption of conservation tillage. Therefore, Monsanto Company requests approval of NK603 and any progeny derived from crosses between NK603 and other commercial corn varieties.