SUMMARY REPORT

RISK ASSESSMENT OF GENETICALLY MODIFIED LEPIDOPTERAN – PROTECTED CORN MON 89034 TO HUMAN AND LIVESTOCK

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 YieldGardVTPro
Gene transfer event: The lepidopteran-protected corn, MON 89034
Introduced trait related to the transformed gene: Lepidopteran insect resistance
The only identified code (if yes): MON-89Ø34-3

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 has developed, through the use of recombinant DNA techniques, MON 89034, a corn product that is protected from damage caused by larval feeding of lepidopteran insect pests. MON 89034 produces the *Bacillus thuringiensis* (*Bt*) Cry1A.105 and Cry2Ab2 proteins that are active against lepidopteran insects. MON 89034 will effectively address a corn grower's need to control a wide spectrum of lepidopteran pests. The combination of the Cry1A.105 and Cry2Ab2 insecticidal proteins in a single plant provides excellent control of lepidopteran insect pests and offers an effective insect resistance management tool.

Corn is the world's leading crop cultivated over an area of about 159 million hectares with a production of about 791 million tones (2007-08). 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%. In Vietnam, corn is grown over an area of approximately one million hectares with total production of about 4.43 million tonnes. The corn crop is susceptible to feeding damage from insect pests resulting in significant economic losses.

The introduction of MON 89034 is expected to provide enhanced benefits for the control of lepidopteran pests of corn compared to single mode of action products. MON 89034 will have a wide spectrum of activity against lepidopteran pests and will strengthen insect resistance management, continue to reduce the potential for mycotoxins in grain, and potentially reduce the refuge acreage required for resistance management purposes. Taken together, adoption of MON 89034 is likely to enhance economic and other benefits (occupational health, flexibility of use, etc) to farmers and improve the quality of grain and the safety of the derived food and feed products.

MON 89034 was produced by *Agrobacterium*-mediated transformation of corn with the PV-ZMIR245 vector, which is a binary vector containing two separate transfer DNA's (2T-DNA). The first T-DNA, designated as T-DNA I, contains the *cry1A.105* and the *cry2Ab2* expression cassettes. The second T-DNA, designated as T-DNA II, contains the *nptII* (neomycin phosphotransferase II) expression cassette. During transformation, both T-DNAs were inserted into the genome. The *nptII* selectable marker gene was used for the selection of transformed cells in the presence of neomycin. A significant proportion of the cells selected for resistance to neomycin due to the presence of T-DNA II will also contain T-DNA I. Once the transgenic cells were identified, the selectable marker gene was no longer needed. Traditional breeding was used to produce plants that only contained the *cry1A.105* and *cry2Ab2* expression cassettes (T-DNA I) and did not contain the *nptII* expression cassette (T-DNA II), thereby, producing marker-free corn MON 89034.

The data and information presented in this summary demonstrate that the foods and feeds derived from MON 89034 are as safe and nutritious as the comparable foods and feeds derived

from conventional corn. This conclusion is based on several lines of evidence. The first is the detailed molecular characterization of the inserted DNA. Results confirm the insertion of a single functional copy of the *cry1A.105* and *cry2Ab2* expression cassettes at a single locus within the genome. The second is a detailed biochemical characterization of the Cry1A.105 and Cry2Ab2 proteins produced in MON 89034. Data demonstrate that the two Cry proteins produced in MON 89034 are equivalent to their counterparts produced by recombinant strains of *Escherichia coli* for use in various safety assessment studies. The third is a thorough assessment of the allergenicity and toxicity potential of the Cry1A.105 and Cry2Ab2 proteins based on extensive studies. The results demonstrate the safety of the Cry1A.105 and Cry2Ab2 proteins due to the lack of allergenic potential and the lack of acute toxicity when ingested, and their similarity to Cry proteins that have a history of safe use. The fourth is the estimation of protein levels and a 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 Cry1A.105 and Cry2Ab2 proteins based on the large margins of exposure (MOEs) obtained. The fifth is the compositional and nutritional assessment of MON 89034 grain and forage, which confirms that MON 89034 is compositionally equivalent to and as safe as conventional corn.

Molecular characterization of MON 89034 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. Results demonstrated that the DNA inserted into the corn genome is present at a single locus and contains one functional copy of the *cry1A.105* and the *cry2Ab2* expression cassettes. All genetic elements were shown to be present in the inserted DNA as expected. However, the *e35S* promoter, which regulates expression of the *cry1A.105* gene, was modified and the Right Border sequence present in PV-ZMIR245 was replaced by a Left Border sequence in MON 89034. There were no other elements, either full length or partial, present other than those associated with the intended insert, and no backbone plasmid DNA or *nptII* sequences were detected. PCR and DNA sequence analyses provided the complete DNA sequence of the insert and confirmed the organization of the elements within the insert. The stability of the integrated DNA and absence of the T-DNA II and backbone sequences in multiple generations of MON 89034 was also confirmed. The heritability of the *cry1A.105* and *cry2Ab2* genes was confirmed by segregation analysis of several generations of MON 89034. These results are consistent with the conclusion of a single active site of insertion that segregates according to the Mendelian laws of genetics.

The donor organism for the Cry1A.105 and Cry2Ab2 proteins, *Bacillus thuringiensis*, has been used commercially in the U.S. for over four decades to produce microbial pesticides. Cry proteins that have a history of safe use since 1958 as active ingredients either in *Bt* microbial pesticides or in biotechnology-derived food and feed crops. Cry1A.105 is a chimeric protein comprised of domains I, II from Cry1Ab and Cry1Ac, the C-terminal portion from Cry1Ac (*Bt*

subsp. *kurstaki*), and domain III from Cry1F (*Bt* subsp. *aizawai*). Domains I and II of Cry1A.105 are 100% identical in amino acid sequence to domains I and II of both Cry1Ab and Cry1Ac, domain III is 99% identical to domain III of Cry1F, and the C-terminal portion is 100% identical to the C-terminal portion of Cry1Ac. The overall amino acid sequence identity to the Cry1Ab, Cry1Ac, and Cry1F proteins is 90.0%, 93.6%, and 76.7 % respectively. *Bt* microbial strains producing Cry1Ac and Cry1Ab, and Cry1F proteins have been used for decades as biopesticides.[1] The Cry1A.105 protein produced in MON 89034 is structurally and functionally similar to Cry1A proteins produced in a number of biotechnology-derived crops (e.g., YieldGard Corn Borer corn, Bollgard® and Bollgard® cotton) that have demonstrated history of safe use.

The Cry2Ab2 protein produced in MON 89034 is derived from the *Bt* subspecies *kurstaki* and its amino acid sequence differs from that of the wild-type protein by a single amino acid. The Cry2Ab2 protein has 88% amino acid sequence identity to the Cry2Aa protein which is present in commercial microbial pest control products such as Dipel® and Crymax®. The Cry2Ab2 proteins produced in MON 89034 and Bollgard II cotton have an identical amino acid sequence. Bollgard II cotton has been on the market since 2003 and there have been no adverse reports regarding its safety.

The assessment of potential allergenicity and toxicity showed there was a reasonable certainty of no harm to mammals from exposure to the Cry1A.105 and Cry2Ab2 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 use, similarity to known toxins or biologically active proteins, and evaluation of acute toxicity to mammals.

As mentioned previously, *Bacillus thuringiensis*, the donor organism for these two Cry proteins, has been used commercially in the U.S. for over four decades to produce microbial pesticides, and there are no confirmed cases of allergic reactions to Cry proteins. Results of extensive bioinformatics assessments using FASTA sequence alignment and eight-amino acid sliding window searches showed that the Cry1A.105 and Cry2Ab2 proteins do not share any amino acid sequence similarities with known allergens, gliadins, glutenins, or protein toxins that have adverse effects to mammals. Assessment of the in vitro digestibility in simulated gastric fluid (SGF) showed that the Cry1A.105 and Cry2Ab2 proteins are rapidly digested, with greater than 95% to 99% of the proteins, respectively, being digested in less than 30 seconds. Proteins that are rapidly digestible have a lower risk of causing allergic reactions or resulting in toxicity when consumed. Mice acute oral toxicity studies demonstrate that the Cry1A.105 and Cry2Ab2 proteins are not acutely toxic and do not cause any adverse effects even at maximum attainable

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[1] Cry1Ab and Cry1Ac are components of the microbial product Dipel®, and a Cry1Ac/Cry1F chimeric protein is a component of the microbial product Lepinox® (Ecogen Inc.).

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dose levels of 2072 and 2198 mg/kg body weight for the Cry1A.105 and Cry2Ab2 proteins, respectively. The independent safety assessment for each of the Cry proteins in mice was considered appropriate and adequate based on the extensive history of safe use of mixtures of Cry proteins present in *Bt* microbial pesticides.

The levels of the Cry1A.105 and Cry2Ab2 proteins estimated in tissues of MON 89034 showed trends that were consistent for exposure calculations and intended uses. Tissues of MON 89034 were collected from field trials conducted at five sites in the U.S. during 2005. Tissues from the different growth stages of the corn plant were collected throughout the growing season and analyzed by enzyme-linked immunosorbent assay (ELISA). The mean Cry1A.105 levels across sites were 520 µg/g dwt in young leaf, 42 µg/g dwt in forage, and 5.9 µg/g dwt in grain. The mean Cry2Ab2 levels across sites were in 180 µg/g dwt in young leaf, 38 µg/g dwt in forage, and 1.3 µg/g dwt in grain. In general, the levels of the two Cry proteins declined over the growing season.

A dietary safety assessment based on Cry1A.105 and Cry2Ab2 protein levels, expected dietary exposure, and the results of acute toxicology tests showed large MOEs for humans and animals. The MOEs for the overall U.S. population were greater than or equal to 199,000 and 981,000 for the Cry1A.105 and Cry2Ab2 proteins, respectively. For children aged 3-5 years old, an age group with the highest corn consumption (body weight basis), the MOEs were greater than or equal to 79,400 and 390,000 for the Cry1A.105 and Cry2Ab2 proteins, respectively. For poultry and livestock, the MOEs ranged between 1,930 – 13,500 and 2,160 – 47,600 for the Cry1A.105 and Cry2Ab2 proteins, respectively. These results are consistent with the extensive safety testing previously conducted for the Cry1A and Cry2A class of proteins.

The EPA further confirmed the safety of the Cry1A.105 and Cry2Ab2 proteins recently when they established temporary exemptions from the requirement of a tolerance for the Cry1A.105 and Cry2Ab2 proteins and the genetic material for their production in corn (40 CFR §174.453 and 40 CFR §174.454, respectively).

Compositional assessment of the grain and forage from MON 89034 demonstrated that it is nutritionally and biologically equivalent to its conventional counterpart, LH198 x LH172. Compositional data on key nutrients, anti-nutrients and other components were collected for the forage and grain from MON 89034 and conventional control corn, grown at five field sites in the U.S. during 2004. Five conventional, commercial corn reference hybrids were also grown at each site, for a total of 15 references. Composition data from the references was used to establish a range of variability described by a 99% tolerance interval for each component analyzed. Statistical comparisons of 61 components from MON 89034 and the control were conducted for the combination of all five sites (i.e., the combined-site) and for each individual site. The overall data set was examined for evidence of biologically relevant changes. Evaluation of the data, including the results of statistical analysis, leads to the conclusion that MON 89034 is compositionally and nutritionally equivalent to conventional corn.

No statistical differences were observed in 58 of 61 combined-site site comparisons made between MON 89034 and the conventional control. The three differences observed were generally small (3.4 – 19.2%), considering the range of natural variability, and the mean levels and ranges of MON 89034 were well within the 99% tolerance intervals for commercial corn. For the individual site analyses, there were no statistical differences that were consistently observed across all sites. Furthermore, the means and ranges of all components from MON 89034 showing a statistical difference were within the 99% tolerance intervals of conventional corn and/or within the International Life Sciences Institute (ILSI) Crop Composition Database.

In conclusion, the data and information presented in this summary demonstrate that the foods and feeds derived from MON 89034 are as safe and nutritious as the comparable foods and feeds derived from conventional corn. This conclusion is based on several lines of evidence including:

1. The detailed molecular characterization of the inserted DNA, which confirmed the presence of a single functional copies of the *cry1A.105* and *cry2Ab2* cassettes, stably integrated at a single locus of the genome;
2. A safety assessment of the Cry1A.105 and Cry2Ab2 proteins, which shows the lack of allergenic potential and acute toxicity when ingested;
3. A dietary safety assessment, which showed and no meaningful risks to human or animal health from dietary exposure to the Cry1A.105 and Cry2Ab2 proteins; and
4. Compositional and nutritional assessments, which demonstrate that the MON 89034 is compositionally equivalent to and as safe as conventional corn.

Therefore, it is concluded that the consumption of MON 89034 and the food and feed derived from it will be as safe and nutritious as that of conventional corn.