SUMMARY REPORT

RISK ASSESSMENT OF GENETICALLY MODIFIED MON 87427 MAIZE WITH TISSUE-SELECTIVE GLYPHOSATE – TOLERANCE FACILITATING THE PRODUCTION OF HYBRID CORN SEED 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: Roundup Hybridization System (RHS)
Gene transfer event: The tissue-selective herbicide-tolerant maize, MON 87427
Introduced trait related to the transformed gene: Tissue-selective glyphosate tolerance
The only identified code (if yes): MON-87427-7

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

Maize MON 87427 contains a *cp4 epsps* gene that encode glyphosate-tolerant EPSPS proteins (CP4 EPSPS) expression in vegetative and female reproductive tissues that confer tolerance to glyphosate, the active ingredient in Roundup agricultural herbicides, the leaves, stalk, and root tissues and tissues that develop into seed or grain and silks.

4. Method of Transformation used:

Agrobacterium-mediated transformation

II. Executive Summary and Overall Conclusions

Monsanto Company has developed biotechnology-derived MON 87427 maize with tissue-selective glyphosate tolerance to facilitate the production of viable hybrid maize seed. MON 87427 produces the same 5-enolpyruvylshikimate-3-phosphate synthase (CP4 EPSPS) protein that is produced in commercial Roundup Ready® crop products, via the incorporation of a *cp4 epsps* coding sequence. CP4 EPSPS protein confers tolerance to the herbicide glyphosate, the active ingredient in the family of Roundup® agricultural herbicides. Tissue-selective expression of CP4 EPSPS protein in MON 87427 facilitates an extension of the use of glyphosate tolerant maize to enable its use as a tool for hybrid maize seed production.

MON 87427 utilizes a specific promoter and intron combination (*e35S-hsp70*) to drive CP4 EPSPS protein expression in vegetative and female reproductive tissues, conferring tolerance to glyphosate in the leaves, stalk, and root tissues and tissues that develop into seed or grain and silks. This specific promoter and intron combination also results in limited or no production of CP4 EPSPS protein in two key male reproductive tissues: pollen microspores, which develop into pollen grains, and tapetum cells that supply nutrients to the pollen. Thus, in MON 87427, male reproductive tissues critical for male gametophyte (pollen) development are not tolerant to glyphosate. This allows glyphosate-treated MON 87427 containing inbred lines to serve as a female parent in the production of hybrid seed. Two glyphosate applications that are made during maize vegetative growth stages ranging from V8 to V13 to inbreds containing MON 87427 will produce a male sterile phenotype through tissue-selective glyphosate tolerance. This will eliminate or greatly reduce the need for detasseling, which is currently used in the production of hybrid maize seed. In a hybrid maize seed production system, the MON 87427 inbred plants, with glyphosate applied during tassel development, will be pollinated by pollen donor (male) plants. This will result in viable hybrid maize seed carrying the gene for tissue-selective glyphosate tolerance. For weed control in both seed and grain production fields, glyphosate may be applied to MON 87427 at vegetative stages as directed on Roundup agricultural product labels, at the same rates used in previously deregulated Roundup Ready® corn 2 events (NK603 and MON 88017).

The benefits of MON 87427 in the production of hybrid seed include:

- *Increased Flexibility in Hybrid Seed Production*: Each year approximately 0.5 M acres used for hybrid maize seed production must be detasseled in order to meet commercial growers' hybrid maize seed needs and to meet established seed purity criteria in the U.S. The critical time period for detasseling is after the tassel has emerged but prior to pollen shed and silk emergence, and encompasses an average 3 - 4 day window. Current detasseling practices may require up to two passes with mechanical detasseling equipment and up to three passes if hand detasseling is used. Further complicating detasseling activity is the logistical planning required for moving enough labor and resources to the designated hybrid seed production fields at the appropriate time. Glyphosate applications made to MON 87427 during the V8 to V13 vegetative growth stages results in the male sterile phenotype. The two glyphosate applications needed to produce the male sterile phenotype would take place during an approximate 14 day

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window within these growth stages; a much longer time period compared to an average 3 – 4 day window between tassel emergence and pollen shed and silk emergence. This timing accounts for significantly improved flexibility in hybrid seed production.

- *Economic Benefits for Hybrid Seed Producers:* Seed manufacturers continually seek ways to improve hybrid seed productivity and reduce the inputs and land area used to produce high quality hybrid seed. Agricultural field labor costs continue to make up a large percentage of total costs to produce seed in the U.S. Compounding this increasing cost is population migration towards urban areas that is shrinking the agricultural labor pool, thus reducing a reliable labor pool for this work. Costs associated with labor recruitment and deployments to perform detasseling are some of the largest cost improvement opportunities in hybrid seed production. MON 87427 will decrease hybrid seed production costs primarily from a reduction in direct and associated labor costs.

Molecular Characterization of MON 87427 Verifies the Integrity and Stability of the Inserted DNA

MON 87427 was developed through *Agrobacterium*-mediated transformation of maize immature embryos from line LH198 × HiII utilizing plasmid vector PV-ZMAP1043. PV-ZMAP1043 contains one T-DNA that is delineated by Left and Right border regions. The T-DNA contains one expression cassette consisting of the *cp4 epsps* coding sequence under the regulation of the *e35S* promoter, the *hsp70* intron, the *CTP2* targeting sequence, and the *nos* 3′ nontranslated region. After transformation, a single plant was selected and increased (MON 87427).

MON 87427 was subjected to an extensive molecular characterization. Southern blot analyses demonstrated that a single copy of the T-DNA sequence from PV-ZMAP1043 was integrated into the maize genome at a single locus. These analyses also demonstrated that there were no additional genetic elements, including backbone sequences, from PV-ZMAP1043 detected, linked or unlinked to the intact T-DNA present in MON 87427. The PCR and DNA sequence analyses performed on MON 87427 confirmed the organization of the elements within the insert, assessed potential rearrangements at the insertion site, and resulted in the determination of the complete DNA sequence of the T-DNA and adjacent maize genomic DNA sequence in MON 87427. Furthermore, Southern blot analysis demonstrated that the T-DNA insert in MON 87427 has been maintained through five breeding generations, thereby confirming the stability of the T-DNA in MON 87427. Finally, results from segregation analyses demonstrated heritability of the insert occurred as expected across multiple generations, which corroborates the molecular insert stability analysis and establishes the genetic behavior of the T-DNA in MON 87427 at a single chromosomal locus.

Data Confirm the Safety of Expression Products in MON 87427

Several Roundup Ready crops that produce the CP4 EPSPS protein have been approved by FDA and submitted to MARD. The CP4 EPSPS protein expressed in MON 87427 is identical to the CP4 EPSPS in other Roundup Ready crops. Results from the protein characterization studies included in this petition confirmed the identity of the MON 87427-produced CP4 EPSPS protein and established the equivalence of the MON 87427-produced protein to the *E. coli*-produced

CP4 EPSPS protein. The safety of CP4 EPSPS proteins present in biotechnology-derived crops has been extensively assessed.

A multistep approach was conducted according to guidelines established by the CODEX Alimentarius Commission and the Organization for Economic Co-operation and Development (OECD) and was used to characterize the CP4 EPSPS protein in MON 87427 resulting from the genetic modification. This detailed assessment confirms the CP4 EPSPS protein is safe for human and animal consumption. The assessment includes: 1) quantification of CP4 EPSPS expression in plant tissues; 2) examination of the similarity of CP4 EPSPS protein to known allergens, toxins or other biologically active proteins known to have adverse effects on humans and animals; 3) evaluation of the digestibility of CP4 EPSPS protein in simulated gastric fluids; 4) documenting the history of safe consumption of CP4 EPSPS protein or its structural and functional homology to proteins that lack adverse effects on human or animal health; and 5) assessment of the potential for allergenicity, toxicity and adverse biological activity of putative polypeptides encoded by the insert. The safety assessment supports the conclusion that dietary exposure to CP4 EPSPS protein derived from MON 87427 poses no meaningful risk to human or animal health.

Food and Feed Safety Assessments of MON 87427 Demonstrate Equivalence to Conventional Crop

Several Roundup Ready crops that produce the CP4 EPSPS protein have been approved by FDA and submitted to MARD. The CP4 EPSPS protein expressed in MON 87427 is identical to the CP4 EPSPS protein in other Roundup Ready crops and the mode of action of CP4 EPSPS protein is well understood. Previous Roundup Ready crops reviewed by the MARD have had no biologically relevant compositional changes identified, and there is no reason to expect the CP4 EPSPS protein in MON 87427 to interact with endogenous metabolites or important nutrients that are present in maize grain or forage.

Detailed compositional analyses in accordance with OECD guidelines were conducted to determine whether levels of key nutrients, anti-nutrients and secondary metabolites in MON 87427 were comparable to levels present in the near-isogenic conventional control and several commercial maize reference hybrids. The maize references were used to establish the natural range of levels of the key nutrients, anti-nutrients, and secondary metabolites in commercial maize hybrids that have a history of safe consumption. Nutrients assessed in this analysis included proximates (ash, carbohydrates by calculation, moisture, protein, and fat), acid detergent fiber (ADF), neutral detergent fiber (NDF), total dietary fiber, amino acids, fatty acids (C8-C22), minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc), and vitamins [folic acid, niacin, A (β-carotene), B1, B2, B6, and E] in the grain, and proximates, ADF, NDF, calcium and phosphorus in forage. The anti-nutrients assessed in grain included phytic acid and raffinose. Secondary metabolites assessed in grain included furfural, ferulic acid, and p-coumaric acid.

Combined-site analyses were conducted to determine statistically significant differences (5% level of significance) between MON 87427 and the conventional control on both forage and grain samples. Statistical results from the combined-site data were reviewed using considerations relevant to safety and/or nutritional value. These considerations included

assessments of: 1) whether the MON 87427 component mean value is within the range of natural variability of that component as represented by the 99% tolerance interval of commercial maize reference hybrids grown concurrently, and 2) assessing the difference within the context of natural variability of commercial maize composition published in the scientific literature and in the International Life Sciences Institute (ILSI) Crop Composition Database.

The levels of assessed components in MON 87427 were compositionally equivalent to the conventional control and within the range of variability of the commercial reference varieties that were grown concurrently. The results demonstrated that the differences observed in the combined-site analysis were not meaningful to food and feed safety or the nutritional quality of MON 87427 maize and support the overall food and feed safety of MON 87427.

Conclusion

All data support the conclusion that food and feed derived from MON 87427 will be as safe and nutritious as food and feed derived from a conventional maize crop.