EXECUTIVE SUMMARY
to
Application to Food Standards Australia New Zealand for the Inclusion of Corn MON87403 in *Standard 1.5.2 - Food Derived from Gene Technology*

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EXECUTIVE SUMMARY

MON 87751 Product Description

Monsanto Company has developed biotechnology-derived maize MON 87403 that has increased ear biomass at an early reproductive stage (R1) compared to conventional control maize. Insertion of the coding region of the Arabidopsis *ATHB17* gene results in production of a truncated ATHB17 protein (ATHB17Δ113) in MON 87403. ATHB17 is a member of the HD-Zip family of plant transcription factors, which are proteins that bind to specific DNA sequences and regulate gene expression. The HD-Zip family of proteins is found broadly across plant species and specific HD-Zip proteins have been shown to play important roles in the modulation of plant growth and development. Increased ear biomass in MON 87403 is associated with increased partitioning of dry matter (photosynthate) from the source (vegetative) tissue to the sink (ear) tissue.

The early reproductive stages in maize are a critical period of maize growth when the maximum ear biomass (sink size) is determined by a combination of genetics and environmental conditions. Dry matter (photosynthate) produced by the plant during reproductive stages is allocated to the ear for its growth after the sink size is established. Thus, ear biomass, which is set during early reproductive stages, is considered an important determinant of future reproductive success such that a larger ear biomass at early reproductive stages is associated with increased grain yield opportunity at harvest.

MON 87403 will be combined with other deregulated biotechnology-derived traits through traditional breeding methods to create commercial products with increased yield opportunity as well as protection against maize insect pests and tolerance to herbicides. These next generation combined-trait maize products will continue to offer growers a broad choice of trait combinations and continued pest control durability.

Molecular Characterization of MON 87403 Verifies the Integrity and Stability of the Inserted DNA

MON 87403 was developed through *Agrobacterium*-mediated transformation of maize immature embryos from inbred line LH244 utilizing plasmid PV-ZMAP5714. PV-ZMAP5714 is approximately 11.7 kb in size and contains three cassettes: one T-DNA, delineated by Left and Right Border regions, contains the *ATHB17* expression cassette, the plasmid backbone contains the *cp4 epsps* selectable marker cassette, and the *aadA* expression cassette. PV-ZMAP5714 employs a tandem T-DNA approach to generate marker-free plants. In this tandem T-DNA approach, a single right border and a single left border were used to achieve separate, unlinked insertions of distinct unlinked T-DNAs, one with the ATHB17 cassette and the other containing the *cp4 epsps* selectable marker gene located in the plasmid backbone. After initial selection of transformants for glyphosate tolerance, the *cp4 epsps* cassette and other genetic material associated with the backbone region as segregated away by conventional breeding and molecular analysis was used to identify plants containing only the intended T-DNA (and not the *cp4 epsps* cassette).

Characterization of the DNA insert in MON 87403 was conducted using a combination of sequencing, PCR, and bioinformatics. The results of this characterization demonstrate that MON 87403 contains one copy of the intended transfer DNA (T-DNA) containing the *ATHB17* expression cassette that is stably integrated at a single locus and is inherited

according to Mendelian principles over multiple generations. These conclusions are based on several lines of evidence:

- Molecular characterization of MON 87403 by Next Generation Sequencing and Junction Sequence Analysis (NGS/JSA) demonstrated that MON 87403 contained a single intended DNA insert. These whole-genome sequence analyses provided a comprehensive assessment of MON 87403 to determine the presence and identity of sequences derived from PV-ZMAP5714 and demonstrated that MON 87403 contained a single T-DNA insert with no detectable backbone sequences.
- Directed sequencing (locus-specific PCR, DNA sequencing and analyses) performed on MON 87403 was used to determine the complete sequence of the single DNA insert from PV-ZMAP5714, the adjacent flanking DNA, and the 5' and 3' insert-to-flank junctions. This analysis confirmed that the sequence and organization of the inserted DNA is identical to the corresponding region in the PV-ZMAP5714 T-DNA. Furthermore, the genomic organization at the insertion site was assessed by comparing the sequences flanking the T-DNA insert in MON 87403 to the sequence of the insertion site in conventional maize. This analysis determined that no major DNA rearrangement occurred at the insertion site in MON 87403 upon DNA integration.
- Generational stability analysis by NGS/JSA demonstrated that the single PV-ZMAP5714 T-DNA insert in MON 87403 has been maintained through five breeding generations, thereby confirming the stability of the T-DNA in MON 87403.
- Segregation analysis corroborated the insert stability demonstrated by NGS/JSA and independently established the nature of the T-DNA as a single chromosomal locus that shows an expected pattern of inheritance.

Taken together, the characterization of the genetic modification in MON 87403 demonstrates that a single copy of the intended T-DNA was stably integrated at a single locus of the maize genome and that no plasmid backbone sequences are present in MON 87403.

ATHB17Δ113 is safe for consumption in food or feed

Characterization of the introduced protein(s) in a biotechnology-derived crop is important to establishing food, feed, and environmental safety. A multistep approach was used to characterize and assess the safety of the ATHB17Δ113 protein expressed in MON 87403 resulting from the genetic modification. The expression level of the ATHB17Δ113 protein in selected tissues of MON 87403 was determined and exposure to humans and animals through diet was evaluated. In addition, the donor organism for the ATHB17Δ113 protein coding sequence, *Arabidopsis thaliana*, is ubiquitous in the environment and is not commonly known for human or animal pathogenicity or allergenicity.

Assessing the safety of the ATHB17Δ113 protein requires a consideration of the predicted exposure to the protein as well as the potential hazard associated with the protein. Data presented demonstrate that the expression of this protein in maize grain, the primary maize product in commerce, is below the limit of detection (LOD) and is extremely low in other tissues tested, including forage, hence exposure to ATHB17Δ113 protein would be negligible. Even though human or animal exposure to ATHB17Δ113 protein in MON 87403 will be extremely low, a multistep approach was still conducted according to the guidelines established by the Codex Alimentarius Commission and OECD to confirm that the ATHB17Δ113 protein present in MON 87403 as a result of the genetic modification does not

pose toxic or allergenic hazards to human or animal health. Bioinformatic searches using the ATHB17Δ113 amino acid sequence to query relevant databases identified homologous sequences from several different widely consumed food plants, including several with long histories of safe consumption such as soybean, rice, maize, tomato, potato, orange, papaya, grape, and cruciferous vegetables. Overall the protein sequence identity of ATHB17Δ113 to homologs in these species ranges as high as 83% (~58-83%), with the highest identity to the homologs in the Brassica species *Brassica rapa* (a species including crops such as turnip and napa cabbage) and *Brassica oleracea* (a species including crops such as cabbage and Brussels sprouts). The amino acid sequence alignment between ATHB17Δ113 and its food crop homologs spans the length of the ATHB17Δ113 protein. Thus ATHB17Δ113 shares sequence identity and structural similarity with proteins present in plants currently consumed, establishing that humans and animals have been and continue to be exposed to this class of proteins and that no adverse effects have been attributed to this class of proteins.

Bioinformatics analysis determined that the ATHB17Δ113 protein lacks structural similarity to known allergens, gliadins, glutenins, or protein toxins. The ATHB17Δ113 protein is rapidly degraded in pepsin and pancreatin, suggesting that the negligible amount of protein expressed is further reduced by proteolysis during ingestion thereby reducing dietary exposure to even lower levels. A mouse gavage study demonstrated no acute oral toxicity with a No Observable Adverse Effect Level (NOAEL) for ATHB17Δ113 of 1335 mg/kg, the highest dose tested. Undetectable levels of ATHB17Δ113 in grain, coupled with no evidence of any toxic or allergenic concerns, supports the conclusion that consumption of the ATHB17Δ113 protein from MON 87403 or its progeny poses no meaningful risk to human or animal health.

Compositional Analysis of MON 87403 Demonstrates Equivalence to the Conventional Crop

Compositional analysis was conducted on grain and forage of MON 87403 grown at eight sites representative of typical agricultural regions for maize production in the U.S. in 2012. The evaluation of MON 87403 followed considerations relevant to the compositional quality of maize as defined by a 2002 OECD maize composition consensus document. Grain samples were analyzed for levels of nutrients including proximates, carbohydrates by calculation, fiber, amino acids, fatty acids, minerals, and vitamins. The anti-nutrients analyzed in grain included phytic acid and raffinose. Secondary metabolites analyzed in grain included furfural, ferulic acid, and p-coumaric acid. Forage samples were analyzed for levels of proximates, carbohydrates by calculation, fiber, and minerals. In total, 78 different components were assayed (nine in forage and 69 in grain).

Of those 78 components, 14 fatty acids, sodium, and furfural had more than 50% of observations below the assay limit of quantitation (LOQ) and were excluded from statistical analysis. Moisture in grain and forage was measured for conversion of components to dry weight, but was not statistically analyzed. Therefore, 60 components were statistically analyzed. The statistical comparisons were based on compositional data combined across all field sites. Statistically significant differences were identified at the 5% level ($\alpha = 0.05$). The compositional data from the reference hybrids were combined across all field sites to calculate a 99% tolerance interval for each assessed component to estimate the natural variability of each component in maize.

Of the 60 components statistically assessed for MON 87403, none of the components showed a significant difference between MON 87403 and the conventional control across all field

sites. These results support the overall conclusion that MON 87403 was not a major contributor to variation in component levels in maize grain and forage and confirmed the compositional equivalence of MON 87403 to the conventional control in levels of these components. These results support the overall food and feed safety of MON 87403.

Conclusion

The data and information presented in this safety summary demonstrate that the food and feed derived from MON 87403 and its progeny are as safe and nutritious as food and feed derived from conventional maize. The food/feed safety of MON 87403 is based on the following lines of evidence:

A detailed molecular characterization of the inserted DNA demonstrated a single copy of the expected T-DNA insert at a single locus within maize genome. The genetic elements are present in the expected order and are inherited following Mendelian principles.

Extensive evaluation of the truncated ATHB17Δ113 protein expressed at low levels in MON 87403 confirms that it is unlikely to be a toxin or allergen.

Based on bioinformatic searches of the T-DNA insert and insert flanking regions, there was no evidence regarding toxicity or allergenicity of putative polypeptides potentially encoded by ORFs generated as a result of the T-DNA insertion in MON 87403.

A comprehensive compositional assessment demonstrated that MON 87403 grain and forage are compositionally equivalent to grain and forage from conventional maize.