EXECUTIVE SUMMARY
to
Application to Food Standards Australia New Zealand for the Inclusion of Maize MON 95379 in *Standard 1.5.2 - Food Derived from Gene Technology*

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Food/Feed Safety and Nutritional Assessment of MON 95379

Insect-protected maize MON 95379 was developed to produce two insecticidal proteins, Cry1B.868 and Cry1Da_7, which protect against feeding damage caused by targeted lepidopteran insect pests. Cry1B.868 is a chimeric protein comprised of domains I and II from Cry1Be (*Bacillus thuringiensis, Bt*), domain III from Cry1Ca (*Bt* subsp. *aizawai*) and C-terminal protoxin domain from Cry1Ab (*Bt* subsp. *kurstaki*). Cry1Da_7 is a modified Cry1Da protein derived from *Bt* subsp. *aizawai*.

MON 95379 was developed to provide growers in South America an additional tool for controlling target lepidopteran maize pests, including fall armyworm resistant to current *Bt* technologies. MON 95379 will be combined through traditional breeding with other deregulated traits to provide protection against both above-ground and below-ground maize pests, as well as herbicide tolerance. These next generation combined-trait maize products will offer broader grower choice, improved production efficiency, increased pest control durability, and enhanced grower profit potential.

Molecular Characterization of MON 95379 Verifies the Integrity and Stability of the Inserted DNA

MON 95379 was produced by *Agrobacterium*-mediated transformation of maize tissue using the transformation vector PV-ZMIR522223. This vector contains a single TDNA (transfer DNA), that is delineated by Right and Left Border regions. The TDNA contains the *cry1B.868* and *cry1Da_7* expression cassettes. The T-DNA that was inserted initially contained a *cp4 epsps* selectable marker cassette flanked by two excision targeting sequences called *lox* sites. After MON 95379 was selected as an acceptable transformant, the selectable marker cassette was excised by crossing MON 95379 with a Cre recombinase expressing line (the "Cre line" was transformed with the vector PVZMOO513642). Subsequently, traditional breeding, segregation, selection, and screening were used to isolate those plants that contained the *cry1B.868* and *cry1Da_7* expression cassettes, and lacked the *cp4 epsps* selectable marker and any sequences from the *cre* gene containing plasmid, PVZMOO513642.

Characterization of the DNA insert in MON 95379 was conducted using a combination of sequencing, polymerase chain reaction (PCR), and bioinformatics. The results of this characterization demonstrate that MON 95379 contains one copy of the intended T-DNA containing the *cry1B.868* and *cry1Da_7* expression cassettes that is stably inherited over multiple generations and segregates according to Mendelian principles. These conclusions are based on several lines of evidence:

- Molecular characterization of MON 95379 by Next Generation Sequencing (NGS) demonstrated that MON 95379 contained a single intended DNA insert. These whole-genome analyses provided a comprehensive assessment of MON 95379 to determine the presence and identity of sequences derived from PV-ZMIR522223 and demonstrated that MON 95379 contained a single T-DNA insert, no detectable backbone or *cp4 epsps* selectable marker sequence from PV-ZMIR522223 or any sequences from PV-ZMOO513642.

- Directed sequencing (locus-specific PCR, DNA sequencing and analyses) performed on MON 95379 was used to determine the complete sequence of the single DNA insert from PV-ZMIR522223, the adjacent flanking genomic DNA, and the 5' and 3' insert-to-flank junctions. This analysis confirmed that the sequence and organization of the DNA is identical to the corresponding region in the PV-ZMIR522223 T-DNA and lacks the *cp4 epsps* selectable marker.

- Furthermore, the genomic organization at the insertion site was assessed by comparing the sequences flanking the T-DNA insert in MON 95379 to the sequence of the insertion site in conventional maize. This analysis determined there was a 160 bp deletion upon T-DNA integration in MON 95379.

- Generational stability analysis by NGS demonstrated that the single PV-ZMIR522223 T-DNA insert in MON 95379 has been maintained through five breeding generations, thereby confirming the stability of the T-DNA in MON 95379.

- Segregation analysis corroborates the insert stability demonstrated by NGS and independently establishes 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 95379 demonstrates that a single copy of the intended T-DNA was stably integrated at a single locus of the maize genome and that no PV-ZMIR522223 plasmid backbone, selectable marker, or PV-ZMOO513642 sequences are present in MON 95379.

Cry1B.868 and Cry1Da_7 are Safe for Consumption in Food or Feed

MON 95379 contains *cry1B.868* and *cry1Da_7* expression cassettes that express Cry1B.868 and Cry1Da_7 proteins. A multistep approach to the safety assessment of the Cry1B.868 and Cry1Da_7 proteins were conducted according to guidelines established by the Codex Alimentarius Commission (Codex Alimentarius, 2009) and OECD. The assessment includes: 1) documenting the history of safe consumption of the expressed protein or its structural and functional homology to proteins that lack adverse effects on human or mammalian health; 2) characterization of the physicochemical and functional properties of the expressed protein; 3) quantification of the proteins' expression levels in plant tissues; 4) examination of the similarity of the expressed protein to known allergens, toxins or other biologically active proteins known to have adverse effects on humans and animals; 5) evaluation of the susceptibility of the expressed protein to the digestive enzymes pepsin and pancreatin; and 6) evaluation of the susceptibility of the expressed proteins' functional activity after heat treatment. The data collected to address these elements collectively supports the conclusion that dietary exposure to Cry1B.868 and Cry1Da_7 proteins derived from MON 95379 pose no meaningful risk to human or animal health.

Compositional Analysis of MON 95379 Demonstrates Equivalence to the Conventional Maize

Safety assessments of biotechnology-derived crops include a comparative safety assessment in which the composition of grain and/or other raw agricultural commodities of the biotechnology-derived crop are compared to the appropriate conventional control that has a history of safe use.

Compositional analysis was conducted on grain and forage of MON 95379 and a conventional control harvested at a total of five sites in the United States during the 2018 season. The evaluation of MON 95379 followed considerations relevant to the compositional quality of maize as defined by the OECD consensus document (OECD, 2002). Harvested grain samples were assessed for moisture and levels of nutrients including proximates (protein, total fat, and ash), amino acids (18 components), fatty acids (22 components), carbohydrates by calculation, fiber (ADF, NDF and TDF), minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium and zinc) and vitamins (vitamin A, vitamin B_1, vitamin B_2, vitamin B_3, vitamin B_6, vitamin B_9 and vitamin E). Grain samples were assessed for levels of other components including anti-nutrients (phytic acid and raffinose) and secondary metabolites (ferulic acid, furfural and p-coumaric acid). Harvested forage samples were assessed for moisture and levels of nutrients including proximates (protein, total fat, and ash), carbohydrates by calculation, fiber (ADF and NDF) and minerals (calcium and phosphorus). In all, 78 different components were analyzed (OECD, 2002). Of the 78 measured components, 15 components (caprylic acid, capric acid, lauric acid, myristic acid, myristoleic acid, pentadecanoic acid, pentadecenoic acid, heptadecanoic acid, heptadecenoic acid, gamma linolenic acid, eicosadienoic acid, eicosatrienoic acid, arachidonic acid, sodium and furfural in grain) had more than 50% of the observations below the assay limit of quantitation (LOQ), and were excluded from statistical analysis. Moisture values for grain and forage were measured for conversion of component from fresh to dry weight, but were not statistically analyzed. Therefore, 61 components were statistically analyzed for all samples. The statistical comparison of MON 95379 and the conventional control was based on compositional data combined across all field sites. Statistically significant differences were identified at the 5% level ($\alpha = 0.05$).

There were no statistically-significant differences ($p<0.05$) for 43 of the 61 components analyzed. There were 18 components in grain (protein, alanine, glutamic acid, isoleucine, leucine, methionine, phenylalanine, serine, threonine, valine, linolenic acid, carbohydrates by calculation, copper, iron, manganese, phosphorus, zinc and vitamin A) that showed a statistically significant difference ($p<0.05$) between MON 95379 and the conventional control. For these components, the mean difference between MON 95379 and the conventional control was less than the conventional control range values. The MON 95379 mean component values were also within the range of values observed in the literature and/or the ILSI-CCDB maize values. Therefore, the statistically-significant differences observed were not biologically meaningful from a food and feed safety perspective. No statistical differences ($p<0.05$) were observed for forage analytes.

The results of the compositional assessment found that there were no compositional differences that were biologically meaningful between MON 95379 and conventional control and support the conclusion that MON 95379 maize is compositionally equivalent to the conventional control. These results support the overall food and feed safety of MON 95379.

Conclusion

The data and information presented in this safety summary support the conclusion that the food and feed derived from MON 95379 and its progeny are as safe and nutritious as food and feed derived from conventional maize. The food and feed safety of MON 95379 is based on the following lines of evidence:

1. A detailed molecular characterization of the inserted DNA demonstrated a single, intact copy of the expected T-DNA insert at a single locus within the MON 95379

genome and absence of plasmid backbone and *cp4 epsps* selectable marker sequence. The genetic elements are present in the expected order and are stably inherited according to Mendelian principles.

2. Extensive evaluation of the Cry1B.868 and Cry1Da_7 proteins demonstrates that they do not pose any meaningful risk to food or feed safety.

3. The comprehensive compositional assessment demonstrated that MON 95379 grain and forage is compositionally equivalent to grain and forage from the conventional control.

The data herein demonstrate that the food and feed derived from MON 95379 and its progeny are as safe and nutritious as food and feed derived from conventional maize.