# EXECUTIVE SUMMARY
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# Application to Food Standards Australia New Zealand for the Inclusion of Maize MON 95275 in *Standard 1.5.2 - Food Derived from Gene Technology*

Submitted by:

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**2 September 2022**

# EXECUTIVE SUMMARY

## Food/Feed Safety and Nutritional Assessment of MON 95275

MON 95275 produces the Mpp75Aa1.1 insecticidal protein derived from *Brevibacillus laterosporus*, the Vpb4Da2[1] insecticidal protein derived from *Bacillus thuringiensis* (*Bt*), and a double-stranded RNA transcript from an inverted repeat sequence designed to match the western corn rootworm (WCR; *Diabrotica virgifera virgifera*) *Snf7* gene (DvSnf7.1). The Mpp75Aa1.1 and Vpb4Da2 proteins combined with DvSnf7.1 RNA provide protection from feeding damage caused by targeted coleopteran insect pests.

MON 95275 was developed to provide growers in North America an additional tool to help control corn rootworm (CRW: *Diabrotica* ssp., Coleoptera; Chrysomelidae) pests, including those that may develop resistance to current *Bt* technologies. MON 95275 will not be offered for commercial use as a stand-alone product, but will be combined through traditional breeding with other deregulated and/or registered traits to provide protection against both above-ground and below-ground maize pests, as well as tolerance to multiple herbicides. These next generation combined-trait maize products will offer broader grower choice, improved production efficiency, and increased pest control durability, and promote a more sustainable agriculture system.

## Molecular Characterization of MON 95275 Verifies the Integrity and Stability of the Inserted DNA

MON 95275 was produced by *Agrobacterium*-mediated transformation of maize tissue using the transformation vector PV-ZMIR525664. This vector contains a single T-DNA (transfer DNA), that is delineated by Right and Left Border regions. The T-DNA, contains the *DvSnf7.1* suppression cassette, and the *mpp75Aa1.1* and *vpb4Da2* 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 95275 was screened and selected as an acceptable transformant, the selectable marker cassette was excised by crossing MON 95275 with a Cre recombinase expressing maize line (the "Cre line" was transformed with the vector PV-ZMOO513642). Subsequently, segregation, selection, and screening were used to isolate those plants that contained the *DvSnf7.1* suppression cassette and the *mpp75Aa1.1* and *vpb4Da2* expression cassettes, and lacked the *cp4 epsps* selectable marker cassette and any sequences from the *cre* gene containing plasmid, PV-ZMOO513642.

Characterization of the DNA insert in MON 95275 was conducted using a combination of sequencing, polymerase chain reaction (PCR), and bioinformatics. The results of this characterization demonstrate that MON 95275 contains one copy of the T-DNA containing the *DvSnf7.1* suppression cassette and the *mpp75Aa1.1* and *vpb4Da2* expression cassettes

[1] The Mpp75Aa1.1 and Vpb4Da2 proteins expressed in MON 95275 were previously known as Cry75Aa1.1 and Vip4Ba1/Vip4Da2, respectively. The reclassification of these two proteins were based on structural homology. Background information on alias names and current names can be found in the following publication: (Crickmore et al., 2021). Historical names of proteins were used in certain final study reports based on the effective date of name change. Historical and current names are considered equivalent.

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 95275 by Next Generation Sequencing (NGS) demonstrated that MON 95275 contained a single T-DNA insert. These whole-genome analyses provided a comprehensive assessment of MON 95275 to determine the presence and identity of sequences derived from PV-ZMIR525664 and demonstrated that MON 95275 contained a single T-DNA insert, no backbone or *cp4 epsps* selectable marker sequence from PV-ZMIR525664 or any sequences from PV-ZMOO513642.

- Directed sequencing (locus-specific PCR, DNA sequencing and analyses) performed on MON 95275 was used to determine the complete sequence of the single DNA insert from PV-ZMIR525664, the adjacent flanking genomic DNA, and the 5' and 3' insert-to-flank junctions. This analysis confirmed that other than a single nucleotide difference in a non-coding intervening sequence, the sequence and organization of the DNA in MON 95275 is identical to the corresponding region in the PV-ZMIR525664 T-DNA and lacks the *cp4 epsps* selectable marker cassette.

- Furthermore, the genomic organization at the insertion site was assessed by comparing the sequences flanking the T-DNA insert in MON 95275 to the sequence of the insertion site in conventional maize. This analysis determined that there was a 746 bp deletion upon T-DNA integration in MON 95275 and a 6 bp co-insert within the 3′ flanking sequence.

- Generational stability analysis by NGS demonstrated that the single PV-ZMIR525664 T-DNA insert in MON 95275 has been maintained through five breeding generations, thereby confirming the stability of the T-DNA in MON 95275.

- 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 95275 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-ZMIR525664 plasmid backbone, selectable marker, or PV-ZMOO513642 sequences are present in MON 95275.

**The Mpp75Aa1.1 and VpbDa4 Proteins, and DvSnf7.1 RNA are Safe for Consumption in Food or Feed**

MON 95275 contains the *DvSnf7.1* suppression cassette and the *mpp75Aa1.1* and *vpb4Da2* expression cassettes that express the DvSnf7.1 transcript and Mpp75Aa1.1 and Vpb4Da2 proteins. A multistep approach to the safety assessment of the MON 95275 Mpp75Aa1.1 and Vpb4Da2 proteins was conducted according to guidance 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 each expressed protein; 3) quantification of each expressed protein expression in plant tissues; 4) examination of the

similarity of each expressed protein to known allergens, toxins or other biologically active proteins known to have adverse effects on humans and other mammals; 5) evaluation of the susceptibility of each expressed protein to the digestive enzymes pepsin and pancreatin; 6) evaluation of the stability of the expressed protein after heat treatment. The safety assessment completed for MON 95275 maize supports the conclusion that exposure to the Mpp75Aa1.1 and Vpb4Da2 proteins derived from MON 95275 would not pose a dietary risk to human or mammalian health.

**Compositional Analysis of MON 95275 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 95275 grown at five sites representative of typical agricultural regions for maize production in the U.S. in 2019. The compositional analysis provided a comprehensive comparative assessment of the levels of key nutrients, anti-nutrients and secondary metabolites in grain and forage of MON 95275 and the conventional control. The analyses followed considerations relevant to the compositional quality of maize as defined by the OECD consensus document (OECD, 2002a). Grain samples were analyzed for moisture and levels of key nutrients including proximates, carbohydrates by calculation, fiber, amino acids, fatty acids, minerals and vitamins. In addition, grain samples were analyzed for levels of the anti-nutrients phytic acid and raffinose and secondary metabolites ferulic acid, furfural and p-coumaric acid. Forage samples were analyzed for moisture and levels of proximates, carbohydrates by calculation, fiber and minerals. In total, 78 different components were assayed (9 in forage and 69 in grain).

Of these, 15 components 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 components to dry weight but were not statistically analyzed. There were no statistically significant differences ($p<0.05$) for 43 of the 61 components analyzed. There were seven components in grain (palmitic acid, stearic acid, oleic acid, linoleic acid, arachidic acid, calcium and vitamin $B_6$) that showed a statistically significant difference ($p<0.05$) between MON 95275 and the conventional control. No statistical differences ($p<0.05$) were observed for forage analytes. For these seven components, the mean difference between MON 95275 and the conventional control were less than the conventional control range values. The MON 95275 mean component values were also within the natural variability of these components as published in the scientific literature on maize composition and/or the AFSI-CCDB.

The results of the compositional assessment found that there were no compositional differences that were biologically meaningful between MON 95275 and conventional control and support the conclusion that MON 95275 maize is compositionally equivalent to the conventional control. These results support the overall food and feed safety of MON 95275.

**Conclusion**

The data and information presented in this safety summary provide a weight of evidence that supports the conclusion that the food and feed derived from MON 95275 and its progeny are as safe and nutritious as food and feed derived from conventional maize. The food and feed safety of MON 95275 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 95275 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 DvSnf7.1 RNA, and Mpp75Aa1.1 and Vpb4Da2 proteins demonstrates that they do not pose any meaningful risk to food or feed safety.

3. The comprehensive compositional assessment demonstrated that MON 95275 grain and forage is compositionally equivalent to grain and forage from appropriate conventional maize.

The data herein demonstrate that the food and feed derived from MON 95275 and its progeny are as safe and nutritious as food and feed derived from conventional maize.