EXECUTIVE SUMMARY
to
Application to Food Standards Australia New Zealand for the Inclusion of Soybean MON 94637 in *Standard 1.5.2 - Food Produced using Gene Technology*

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EXECUTIVE SUMMARY

Food Safety and Nutritional Assessment of MON 94637

Insect-protected soybean MON 94637 was developed to produce two insecticidal proteins, Cry1A.2 and Cry1B.2, which protect against feeding damage caused by targeted lepidopteran insect pests. Cry1A.2 is a chimeric protein comprised of domains I from Cry1Ah, domain II and the C-terminal domain from Cry1Ac, and domain III from Cry1Ca. Cry1B.2 is a chimeric protein comprised of domains I and II from Cry1Be, domain III from Cry1Ka2, and the C-terminal domain from Cry1Ab.

MON 94637 was developed to provide growers in South America an additional tool for controlling target lepidopteran soybean pests, including soybean looper and velvetbean caterpillar. MON 94637 will be combined through traditional breeding with other deregulated traits to provide protection against feeding damage caused by lepidopteran pests, as well as tolerance to multiple herbicides. The production of the Cry1A.2 and Cry1B.2 proteins in MON 94637 provides two additional *Bt* Cry insecticidal proteins for managing potential insect resistance and prolonging product durability (Chen et al., 2021).

There are no plans currently to commercialize MON 94637 in the U.S., but it is intended to be cultivated in small-scale breeding, testing, and seed increase nurseries to develop seed for future products in South America.

Molecular Characterization of MON 94637 Verifies the Integrity and Stability of the Inserted DNA

MON 94637 was produced by *Agrobacterium*-mediated transformation of soybean using the transformation plasmid vector PVGMIR527237. This plasmid vector contains two T-DNAs (transfer DNAs), delineated by Right and Left Border regions. TDNA I contains the *cry1A.2* and *cry1B.2* expression cassettes and T-DNA II contains the *splA* and *aadA* expression cassettes.

Characterization of the DNA insert in MON 94637 was conducted using a combination of sequencing, polymerase chain reaction (PCR), and bioinformatics. The results of this characterization demonstrate that MON 94637 contains one copy of the intended transfer DNA (TDNA I), containing the *cry1A.2* and *cry1B.2* expression cassettes, which is stably inherited over multiple generations and segregates according to Mendelian principles. The results of this characterization also confirm that T-DNA II and plasmid backbone are not present in MON 94637. These conclusions are based on several lines of evidence:

- Molecular characterization of MON 94637 by Next Generation Sequencing (NGS) demonstrated that MON 94637 contained a single copy of T-DNA I insert. These whole-genome analyses provided a comprehensive assessment of MON 94637 to determine the presence and identity of sequences derived from the transformation plasmid, PV-GMIR527237, and demonstrated that MON 94637 contained a single copy of T-DNA I insert with no detectable plasmid backbone or T-DNA II sequences.

- Directed sequencing (locus-specific PCR, DNA sequencing and analyses) performed on MON 94637 was used to determine the complete sequence of the single DNA insert from PV-GMIR527237, the adjacent

flanking DNA, and the 5' and 3' insert-to-flank junctions. This analysis confirmed that the sequence and organization of the DNA insert is identical to the corresponding region in the PV-GMIR527237 T-DNA I.

- Furthermore, the genomic organization at the insertion site in MON 94637 was assessed by comparing the sequences flanking the T-DNA I insert in MON 94637 to the sequence of the insertion site in conventional soybean. This analysis determined that no major DNA rearrangement occurred at the insertion site in MON 94637 upon DNA integration, although a 14 base deletion was observed.

- Generational stability analysis by NGS demonstrated that the single PVGMIR527237 T-DNA I insert in MON 94637 has been maintained through five breeding generations, thereby confirming the stability of the T-DNA I insert in MON 94637.

- Segregation data confirm that the inserted T-DNA I segregated following Mendelian inheritance patterns, which corroborates the insert stability demonstrated by NGS and independently establishes the nature of the T-DNA I insert as a single chromosomal locus.

Taken together, the characterization of the genetic modification in MON 94637 demonstrates that a single copy of the intended T-DNA I was stably integrated at a single locus of the soybean genome and that no PV-GMIR527237 plasmid backbone or T-DNA II sequences are present in MON 94637.

Cry1A.2 and Cry1B.2 are Safe for Consumption in Food

The Cry1A.2 and Cry1B.2 proteins expressed in MON 94637 are plant-incorporated protectants (PIPs) regulated by the U.S. Environmental Protection Agency (EPA). In accordance with FDA's 1992 policy on foods derived from new plant varieties, and additional guidance in 2006, it is the purview of the EPA to assess the food safety of PIPs. The data for the safety assessment of the Cry1A.2 and Cry1B.2 proteins in MON 94637 will be submitted in an application to EPA for a permanent exemption from the requirement to establish a tolerance for Cry1A.2 and Cry1B.2 in or on food commodities of soybean.

Studies have been completed to assess the safety of the Cry1A.2 and Cry1B.2 proteins present in MON 94637 in accordance with guidelines established by the Codex Alimentarius Commission, OECD, and EPA, and the principles and guidance of the FDA's 1992 policy on foods from new plant varieties. The weight-of-evidence of these analyses indicates that the Cry1A.2 and Cry1B.2 proteins do not pose a food safety concern.

Compositional Analysis of MON 94637 Demonstrate Equivalence to the Conventional Soybean

Safety assessments of biotechnology-derived crops follow the comparative safety assessment process in which the composition of grain and forage 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 94637 grown at five sites representative of typical agricultural regions for soybean production in the U.S. in 2021. The compositional analysis provided a comprehensive comparative assessment of the levels of key nutrients, anti-nutrients and isoflavones of MON 94637 and the conventional control. The analyses followed considerations relevant to the compositional quality of soybean as defined by the OECD consensus document (OECD, 2012). Grain samples were analyzed for moisture and levels of key nutrients including proximates, carbohydrates by calculation, fiber, amino acids, fatty acids, minerals and vitamins. Nutrients assessed in forage included proximates, carbohydrates by calculation, and fiber. In addition, grain samples were analyzed for levels of several anti-nutrients and isoflavones.

The results of the compositional assessment found that there were no compositional differences that were biologically meaningful between MON 94637 and conventional control and support the conclusion that MON 94637 soybean is compositionally equivalent to the conventional control. These results support the overall food safety of MON 94637.

Conclusion

The data and information presented in this safety summary provide a weight of evidence that supports the conclusion that the food derived from MON 94637 and its progeny are as safe and nutritious as food derived from conventional soybean. The food safety of MON 94637 is based on the following lines of evidence:

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

2. Based on bioinformatics searches of the T-DNA insert and insert flanking regions, there was no evidence for concern regarding health implications of putative polypeptides potentially encoded by ORFs generated as a result of the T-DNA insertion in MON 94637.

3. The comprehensive compositional assessment demonstrated that MON 94637 grain and forage is compositionally equivalent to grain and forage from conventional soybean.

Therefore, the consumption of MON 94637 and its progeny, and the food derived from it will be fully consistent with FDA's Policy (U.S. FDA, 1992) and in compliance with all applicable requirements of the Federal Food, Drug and Cosmetic Act. The data herein demonstrate that the food derived from MON 94637 and its progeny are as safe and nutritious as food derived from conventional soybean.

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