EXECUTIVE SUMMARY
to
Application to Food Standards Australia New Zealand for the Inclusion of Canola MON 94100 in *Standard 1.5.2 - Food Derived from Gene Technology*

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Food/Feed Safety and Nutritional Assessment of MON 94100

Herbicide-tolerant canola, MON 94100, was developed, that is tolerant to dicamba (3,6-dichloro-2-methoxybenzoic acid) herbicide. MON 94100 contains a demethylase gene from *Stenotrophomonas maltophilia* that expresses a dicamba mono-oxygenase (DMO) protein to confer tolerance to dicamba herbicide.

MON 94100 will provide canola growers a tool for effective and sustainable weed management, including control of glyphosate resistant weeds. The best management practices for minimizing the development of herbicide-resistant weeds are built on the concepts of implementing diversified weed management programs, which includes using multiple herbicides with different modes of action either in mixtures, sequences, or in rotation.

MON 94100 will be combined with other authorized biotechnology-derived traits (e.g., Roundup Ready) through traditional breeding methods to create commercial products with tolerance to multiple herbicides. These next-generation, combined-trait canola products will continue to offer broader grower choice in herbicide options available to them and support continued weed control durability.

Molecular Characterization of MON 94100 Verifies the Integrity and Stability of the Inserted DNA

MON 94100 was produced by *Agrobacterium*-mediated transformation of canola tissue using the transfer DNA (T-DNA) transformation vector PV-BNHT508701. This plasmid vector contains two T-DNAs, which are delineated by Right and Left Border regions. T-DNA I contains the *dmo* expression cassette and T-DNA II contains the *splA* and *aadA* selectable marker expression cassettes. Although both *aadA* and *splA* selectable marker expression cassettes were present in PV-BNHT508701, selection for transformants was based on spectinomycin resistance conferred by *aadA*. *SplA* was used as a visual demonstration that T-DNA II is present or absent, and was not used as a selectable marker. Subsequently, traditional breeding, segregation, selection and screening were used to isolate those plants that contain the *dmo* expression cassette and lack the *splA* and *aadA* cassettes.

Characterization of the DNA insert in MON 94100 was conducted using a combination of sequencing, PCR, and bioinformatics. The results of this characterization demonstrate that MON 94100 contains one copy of the intended transfer DNA (T-DNA I) containing the *dmo* expression cassette that is stably inherited over multiple generations and segregates according to Mendelian principles. The results of this characterization also confirm that T-DNA II and the plasmid vector backbone are not detectable. These conclusions are based on several lines of evidence:

- Molecular characterization of MON 94100 by Next Generation Sequencing (NGS) demonstrated that MON 94100 contained a single intended DNA insert. These whole-genome sequence analyses provided a comprehensive assessment of MON 94100 to determine the presence and identity of sequences derived from PV-BNHT508701 and demonstrated that MON 94100 contained a single T-DNA I insert with no detectable backbone or T-DNA II sequences.

- Directed sequencing (locus-specific PCR, DNA sequencing and analyses) performed on MON 94100 was used to determine the complete sequence of the single DNA insert from PV-BNHT508701, 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-BNHT508701 T-DNA I. Furthermore, the genomic organization at the insertion site in MON 94100 was assessed by comparing the sequences flanking the T-DNA I insert in MON 94100 to the sequence of the insertion site in conventional canola. This analysis determined that no major DNA rearrangement occurred at the insertion site in MON 94100 upon DNA integration. This analysis identified an 8 bp deletion at the site of insertion that occurred during integration of the T-DNA I sequences.

- Generational stability analysis by NGS demonstrated that the single PV-BNHT508701 T-DNA I insert in MON 94100 has been maintained through five breeding generations, thereby confirming the stability of the T-DNA I in MON 94100.

- Segregation analysis corroborates the insert stability demonstrated by NGS and independently establishes the nature of the T-DNA I as a single chromosomal locus that shows an expected pattern of inheritance.

Taken together, the characterization of the genetic modification in MON 94100 demonstrates that an intact single copy of the intended T-DNA I was stably integrated at a single locus of the canola genome and that no plasmid backbone or T-DNA II sequences are present in MON 94100.

DMO Protein is Safe for Consumption in Food or Feed

MON 94100 contains a *dmo* expression cassette that expresses a single MON 94100 DMO precursor protein that is post-translationally processed during the chloroplast targeting process into two forms of the DMO protein, referred to as DMO and DMO+27. MON 94100 DMO and DMO+27 are identical to the DMO and DMO+27 proteins expressed in MON 87708 soybean for which the characterization and safety assessment was reviewed and approved by FSANZ in 2012 (A1063). Both forms of the proteins will collectively be referred to as "MON 94100 DMO protein" and distinctions will only be made where necessary. The two forms of DMO proteins expressed in MON 94100 are identical to the previously reviewed forms of MON 87708 DMO proteins in structure of the catalytic site, function, immunoreactivity and substrate specificity. Therefore, acute toxicity, digestibility and heat susceptibility studies described on MON 87708 DMO proteins in A1063 are directly applicable to the safety assessment of DMO proteins expressed in MON 94100.

Further, the expression levels of DMO protein were determined and shown to be present at very low levels in the harvested grain of MON 94100. The physicochemical and functional characteristics of the MON 94100 DMO protein was determined and shown to be equivalent to grain-produced MON 87708 DMO used in protein safety studies. The donor organism for the MON 94100 DMO protein coding sequence, *S. maltophilia*, is ubiquitous in the environment and is not commonly known for allergenicity and human or animal pathogenicity. A bioinformatics analysis confirmed that the MON 94100 DMO protein lacks

structural similarity to known allergens and toxins, or other proteins known to have adverse effects on mammals. The MON 94100 DMO protein was rapidly degraded in the presence of pepsin and pancreatin under physiological conditions. The MON 94100 DMO protein demonstrated substantial loss of activity upon heating at temperatures well below standard food processing temperatures and therefore, it is reasonable to conclude that they would not be consumed as an active protein. The MON 94100 DMO protein demonstrated no oral toxicity in mice at the level tested and the overall animal exposure as a percent of total protein is demonstrated to be very low.

Taken together, similar to previous conclusions on MON 87708 by FSANZ, this safety assessment reaffirms the conclusions that the DMO protein expressed in MON 94100 canola or its progeny, and the consumption of food and feed products derived from MON 94100 do not pose a health risk to humans or animals. Therefore, MON 94100 canola is as safe as canola currently on the market.

In addition, DMO proteins that are highly similar to those produced in MON 94100 are also present in MON 88701 cotton and MON 87419 maize (Wang et al., 2016), which had been approved by FSANZ in 2014 (A1080) and 2016 (A1118), respectively. The safety of DMO protein has also been favorably assessed following extensive reviews by regulatory agencies in at least 15 different countries.

Compositional Analysis of MON 94100 Demonstrates Equivalence to the Conventional Canola

Compositional analysis was conducted on grain of MON 94100 and a conventional control grown at five sites in the United States and Canada during the 2018 season. The evaluation of MON 94100 focused on key nutrients and anti-nutrients of canola grain as defined by the OECD consensus document (OECD, 2011). Harvested grain samples were assessed for moisture and levels of key nutrients including proximates (protein, total fat and ash), amino acids (18 components), fatty acids (21 components), carbohydrates by calculation, fiber (acid detergent fiber (ADF) and neutral detergent fiber (NDF), minerals (calcium and phosphorus) and vitamins (vitamin E and vitamin K_1). Grain samples were also assessed for levels of anti-nutrients (phytic acid, tannins, sinapine, total glucosinolates, total alkyl glucosinolates and total indolyl glucosinolates). Of the 56 measured components, 10 components (caproic acid, caprylic acid, capric acid, lauric acid, myristic acid, heptadecanoic acid, heptadecenoic acid, eicosadienoic acid, erucic acid and docosadienoic acid) had more than 50% of the observations fall below the assay limit of quantitation (LOQ) and were excluded from statistical analyses. Moisture values for grain were measured for conversion of components from fresh to dry weight, but were not statistically analyzed. Therefore, 45 components were statistically analyzed. The statistical comparison of MON 94100 and the conventional control was based on compositional data combined across all field sites. Statistically significant differences were evaluated at the 5% level ($\alpha = 0.05$).

There were no statistically-significant differences ($p<0.05$) for 44 of the 45 components analyzed. There was one component (the anti-nutrient sinapine) that showed a statistically-significant difference ($p<0.05$) between MON 94100 (0.75% dw) and the conventional control (0.73% dw). For this component, the mean difference between MON 94100 and the conventional control was less than the range of the conventional control values. The MON 94100 mean component values were also within the range of values observed in the literature and/or the ILSI-CCDB values.

These data indicated that the statistically significant difference observed was not biologically meaningful from a food and feed safety perspective. These results support the conclusion that MON 94100 canola is compositionally equivalent to the conventional control in levels of key nutrients and anti-nutrients in grain.

Conclusion

The data and information presented in this safety summary support the conclusion that the food and feed derived from MON 94100 and its progeny are as safe and nutritious as food and feed derived from conventional canola. The food/feed safety of MON 94100 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 I insert at a single locus within the canola genome. The genetic elements are present in the expected order and are stably inherited following Mendelian principles.

2. Extensive evaluation of the DMO protein demonstrates that it does not pose any meaningful risk to food or feed safety.

3. The comprehensive compositional assessment demonstrated that MON 94100 grain is compositionally equivalent to grain from conventional canola.

The data herein demonstrate that the food and feed derived from MON 94100 and its progeny are as safe and nutritious as food and feed derived from conventional canola.