EXECUTIVE SUMMARY
to
Application to Food Standards Australia New Zealand for the Inclusion of Glyphosate Tolerance Canola MON 88302 in Standard 1.5.2 - Food Derived from Gene Technology

Submitted by:

Monsanto Australia Limited
Level 12 / 600 St Kilda Road,
Melbourne Victoria 3004

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EXECUTIVE SUMMARY

MON 88302 Product Description

Monsanto Company has developed a second-generation glyphosate-tolerant canola product, MON 88302, designed to provide growers with improved weed control through tolerance to higher rates of glyphosate and greater flexibility for glyphosate herbicide application. MON 88302 produces the same 5-enolpyruvylshikimate-3-phosphate synthase (CP4 EPSPS) protein that is produced in commercial Roundup Ready® crop products, via the incorporation of a *cp4 epsps* coding sequence. The CP4 EPSPS protein confers tolerance to the herbicide glyphosate, the active ingredient in the family of Roundup agricultural herbicides.

MON 88302 utilizes a *FMV/Tsf1* chimeric promoter sequence to drive CP4 EPSPS expression in different plant tissues including pollen. By virtue of CP4 EPSPS expression in pollen, MON 88302 provides tolerance to glyphosate during the sensitive reproductive stages of growth, and enables the application of glyphosate at higher rates up to first flower with no detectable impact to male fertility.

Weed competition can be a major limiting factor in canola production leading to significant yield reductions. Certain weeds, such as Canada thistle and, in Australia, silver grass, wild radish and turnip are known to be particularly important to control in Australian canola production. For example, studies have demonstrated that only ten Canada thistle plants per square meter have resulted in 10% yield loss while forty plants per square meter have resulted in over 50% yield loss. While glyphosate is highly effective against the majority of annual and perennial grasses and broad-leaf weeds, the higher glyphosate rates and extended timing for applications possible with MON 88302 will enable better control of difficult to manage weeds. Use of MON 88302 will provide (1) an opportunity to control weeds if glyphosate application is delayed due to weather or equipment failure; (2) the ability to apply glyphosate according to the weed development stage instead of the canola developmental stage; (3) enhanced protection of canola plants at more advanced development stages at the time of glyphosate application and (4) better control of weeds such as silver grass, wild radish, turnip, Canada thistle, dandelion, common lambsquarters, kochia, smartweed and wild buckwheat. Use of MON 88302 will provide growers with the opportunity to ensure weeds that may impact yields are removed at the optimal time.

Molecular Characterization of MON 88302 Verifies the Integrity and Stability of the Inserted DNA

MON 88302 was developed through *Agrobacterium*-mediated transformation of hypocotyls from canola variety Ebony utilizing plasmid vector PV-BNHT2672. PV-BNHT2672 contains one T-DNA that is delineated by Left and Right Border regions. The T-DNA contains the *cp4 epsps* coding sequence under the control of the *FMV/Tsf1* chimeric promoter, the *Tsf1* leader and intron sequences, and the *E9* 3′ untranslated region. The chloroplast transit peptide CTP2 directs transport of the CP4 EPSPS protein to the chloroplast and is derived from *CTP2* target sequence of the *Arabidopsis thaliana shkG* gene. After transformation and subsequent rounds of self-pollination, homozygous R_2 plants containing only a single T-DNA insertion were identified resulting in production of glyphosate-tolerant canola MON 88302.

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Molecular characterization by Southern blot analyses determined that MON 88302 contains one copy of the T-DNA at a single integration locus and all genetic elements are present. These data also demonstrated that MON 88302 does not contain detectable backbone sequences from the plasmid vector. The complete DNA sequence of the insert and adjacent genomic DNA sequences in MON 88302 confirmed the integrity of the inserted *cp4 epsps* expression cassette within the inserted sequences and identified the 5′ and 3′ insert-to-genomic DNA junctions. Southern blot analysis demonstrated that the insert in MON 88302 has been maintained over multiple generations of breeding, thereby confirming the stability of the insert. Further, results from segregation analyses show inheritance and stability of the insert were as expected across multiple generations, which corroborates the molecular insert stability analysis and establishes the genetic behavior of the T-DNA in MON 88302 at a single chromosomal locus.

Data Confirm the Safety of Expression Product in MON 88302

The safety of CP4 EPSPS protein present in biotechnology-derived crops has been extensively assessed. Numerous Roundup Ready crops including Roundup Ready soybean, Roundup Ready 2 Yield soybean, Roundup Ready corn 2, Roundup Ready canola, Roundup Ready sugar beet, Roundup Ready cotton, Roundup Ready Flex cotton and Roundup Ready alfalfa that produce the CP4 EPSPS protein have been reviewed by Food Standards of Australia and New Zealands (FSANZ). The CP4 EPSPS protein expressed in MON 88302 is identical to the CP4 EPSPS in other Roundup Ready crops.

A multistep approach was used to characterize the CP4 EPSPS protein present in MON 88302 as a result of the genetic modification. These steps include: 1) documentation of the history of safe use of the CP4 EPSPS protein and its homology with proteins that lack adverse effects on human or animal health; 2) characterization of the physicochemical and functional properties of CP4 EPSPS; 3) quantification of CP4 EPSPS expression in plant tissues; 4) examination of the similarity of CP4 EPSPS to known allergens, 5) evaluation of the digestibility of CP4 EPSPS in simulated gastrointestinal fluids; 6) evaluation of the stability of the CP4 EPSPS protein in response to typical food/feed preparation conditions such as heat treatment; 7) examination of the similarity of CP4 EPSPS to known toxins or other biologically active proteins known to have adverse effects on mammals; 8) investigation of potential mammalian toxicity through an animal assay; and 9) examination of the similarity of putative polypeptides encoded by the insert and flanking sequences to known allergens and toxins, or other biologically active proteins known to have adverse effects on mammals. The safety assessment supports the conclusion that dietary exposure to CP4 EPSPS protein derived from MON 88302 poses no meaningful risk to human or animal health.

Food and Feed Safety Assessments of MON 88302 Demonstrate Equivalence to the Conventional Crop

Several Roundup Ready crops that produce the CP4 EPSPS protein have been reviewed by FSANZ. The CP4 EPSPS protein expressed in MON 88302 is identical to the CP4 EPSPS protein in other Roundup Ready crops and the mode of action of the CP4 EPSPS protein is well understood. Previous Roundup Ready crops reviewed by FSANZ have had no biologically relevant compositional changes identified, and there is no reason to expect expression of the CP4 EPSPS protein in MON 88302 would affect nutritionally important nutrients, toxicants, and anti-nutrients present in seed from this new product.

Safety assessments of biotechnology-derived crops typically include comparisons of the composition of grain and/or other raw agricultural commodities of the biotechnology-derived

crop to that of conventional counterparts. Compositional assessments were performed using the principles and analytes outlined in crop-specific OECD consensus documents, in this case for canola composition.

Compositional analysis comparing MON 88302 to the conventional control variety (Ebony) and commercial conventional reference varieties demonstrated that MON 88302 is compositionally equivalent to conventional canola. The background genetics of the conventional control were similar to that of MON 88302, but did not contain the *cp4 epsps* expression cassette. The commercial reference varieties were used to define the natural variability of key nutrients, toxicants, and anti-nutrients in canola varieties that have a history of safe consumption. Nutrients assessed in this analysis included proximates (ash, carbohydrates by calculation, moisture, protein, and total fat), fibers (acid detergent fiber [ADF], neutral detergent fiber [NDF], and total dietary fiber [TDF]), amino acids (18 components), fatty acids (FA; C8-C24,), vitamin E (α-tocopherol), and minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc) in seed. The toxicants assessed in seed included erucic acid and glucosinolates (alkyl glucosinolates [including 3-butenyl, 4-pentenyl, 2-hydroxy-3-butenyl, and 2-hydroxy-4-pentenyl glucosinolates], indolyl glucosinolates [including 3-indolylmethyl and 4-hydroxy-3-indoylmethyl], and total glucosinolates). The anti-nutrients assessed in seed included phytic acid, sinapine (as sinapic acid), and total tannins (as the sum of soluble and insoluble tannin fractions).

Combined-site analyses were conducted to determine statistically significant differences ($\alpha = 0.05$) between MON 88302 and the conventional control seed samples. Statistical results from the combined-site data were evaluated using considerations relevant to the safety and nutritional quality of MON 88302 when compared to the conventional control. Considerations used to assess the relevance of each combined-site statistically significant difference included: 1) the relative magnitude of the difference in the mean values of nutrient, toxicant, and anti-nutrient components between MON 88302 and the conventional control; 2) whether the MON 88302 component mean value is within the range of natural variability of that component as represented by the 99% tolerance interval of the commercial reference varieties grown concurrently in the same trial; 3) evaluation of the reproducibility of the statistically significant ($\alpha = 0.05$) combined-site component differences at individual sites, and 4) an assessment of the differences within the context of natural variability of commercial canola composition published in the scientific literature. If statistically significant differences detected in the individual site analyses were not observed in the combined-site analysis, they were not considered further for the compositional assessment of safety.

The levels of assessed components in MON 88302 were compositionally equivalent to the conventional control and within the range of variability of commercial reference varieties grown concurrently in the same field trial. The genetic modification in MON 88302 does not meaningfully impact seed composition and therefore the food and feed safety and nutritional quality of this product is comparable to conventional canola with a history of safe consumption.

Traditional canola processing is described in section A2(b)(iv) of the application. The processing of MON 88302 is not expected to be any different from that of conventional canola. As summarized above, detailed compositional analyses of key components of MON 88302 have been performed and have demonstrated that MON 88302 is compositionally equivalent to conventional canola. Additionally, the mode of action of the CP4 EPSPS protein, as described in section B2(a) of the application, is well understood, and

there is no reason to expect interactions of this protein with important nutrients or endogenous toxicants that may be present in canola. Therefore, when MON 88302 is used on a commercial scale as a source of food or feed, these products are not expected to be different from the equivalent foods or feeds originating from conventional canola.

Conclusion

All data and information strongly support the conclusion that food and feed derived from MON 88302 and its progeny will be as safe and nutritious as food and feed derived from conventional canola.