



EXECUTIVE SUMMARY
to
Application to Food Standards Australia New Zealand
for the Inclusion of Soybean MON 94313
in Standard 1.5.2 - Food Derived from Gene Technology

Submitted by:

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EXECUTIVE SUMMARY

Food/Feed Safety and Nutritional Assessment of MON 94313

Bayer has developed herbicide tolerant soybean MON 94313 that is tolerant to the herbicides glufosinate (2-amino-4-(hydroxymethylphosphinyl) butanoic acid), dicamba (3,6-dichloro-2-methoxybenzoic acid), 2,4-D (2,4-dichlorophenoxyacetate), and mesotrione (2-[4-(methylsulfonyl)-2-nitrobenzoyl]-1,3-cyclohexanedione). MON 94313 soybean contains the phosphinothricin N-acetyltransferase (*pat*) gene from *Streptomyces viridochromogenes* that expresses the PAT protein to confer tolerance to glufosinate herbicide, a demethylase (*dmo*) gene from *Stenotrophomonas maltophilia* that expresses a dicamba mono-oxygenase (DMO) protein to confer tolerance to dicamba herbicide, the *ft_t.1* gene, a modified version of the R-2,4-dichlorophenoxypropionate dioxygenase (*RdpA*) gene from *Sphingobium herbicidovorans* that expresses a FOPs and 2,4-D dioxygenase protein (FT_T.1) to confer tolerance to 2,4-D herbicide, and the *TDO* gene from *Oryza sativa* that expresses the triketone dioxygenase (TDO) protein to confer tolerance to mesotrione herbicide.

MON 94313 soybean will offer growers multiple choices for effective weed management including tough-to-control and herbicide-resistant broadleaf and grass weeds. The flexibility to use combinations of any of these four herbicides representing multiple modes-of-action provides an effective and more durable weed management system for soybean production. The best management practices for minimizing the development of herbicide resistant weeds are built on the concept of implementing diversified weed management programs, which includes using multiple herbicides with different modes of action either in mixtures, sequences or in rotation, and other recommended integrated weed management principles.

MON 94313 soybean will be combined, through traditional breeding methods, with other authorized events (e.g., glyphosate-tolerant trait). MON 94313 soybean combined with glyphosate-tolerant soybean systems through traditional breeding will provide: 1) an opportunity for an efficient, effective weed management system for hard-to-control and herbicide-resistant weeds; 2) a flexible system with multiple herbicide modes-of-action for in-crop application in current soybean production systems; 3) an opportunity to delay selection for further resistance to glyphosate or other herbicides that are important in crop production; 4) excellent crop tolerance to dicamba, glufosinate, 2,4-D, mesotrione and glyphosate; and 5) additional tools to enhance weed management systems necessary to maintain or improve soybean yield and quality to meet the growing needs of the food, feed, and industrial markets.

Molecular Characterization of MON 94313 Verifies the Integrity and Stability of the Inserted DNA

Creation of MON 94313 was accomplished using a binary vector system with two transfer DNAs, one containing the expression cassettes for the herbicide tolerance traits (T-DNA I) and a second with markers for selection of transformed plants (T-DNA II). Characterization of the DNA insert in MON 94313 was conducted using a combination of sequencing, polymerase chain reaction (PCR), and bioinformatics. The results of this characterization demonstrate that MON 94313 contains one copy of the intended transfer DNA (T-DNA I) containing the *dmo*, *pat*, *ft_t.1*, and *TDO* expression cassettes that is stably inherited over multiple generations and segregates according to Mendelian principles. The results of this

characterization also confirm that T-DNA II is not present. These conclusions are based on several lines of evidence:

- Molecular characterization of MON 94313 by Next Generation Sequencing (NGS) demonstrated that MON 94313 contained a single intended DNA insert. These whole-genome analyses provided a comprehensive assessment of MON 94313 to determine the presence and identity of sequences derived from the transformation vector PV-GMHT529103 and demonstrated that MON 94313 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 94313 was used to determine the complete sequence of the single DNA insert from PV-GMHT529103, 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-GMHT529103 T-DNA I.

- Furthermore, the genomic organization at the insertion site in MON 94313 was assessed by comparing the sequences flanking the T-DNA I insert in MON 94313 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 94313 upon DNA integration, although a 40 bases deletion was observed.

- Generational stability analysis by NGS demonstrated that the single PV-GMHT529103 T-DNA I insert in MON 94313 has been maintained through five breeding generations, thereby confirming the stability of the T-DNA I insert in MON 94313.

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

Taken together, the characterization of the genetic modification in MON 94313 demonstrates that a single copy of the intended T-DNA I insert was stably integrated at a single locus of the soybean genome and that no plasmid backbone or T-DNA II sequences are present in MON 94313.

DMO, PAT, FT_T.1 and TDO Proteins are Safe for Consumption in Food or Feed

MON 94313 contains a dmo expression cassette that expresses a single MON 94313 DMO precursor protein that is post-translationally processed during the chloroplast targeting process. DMO proteins highly similar to that produced in MON 94313 are also present in other Bayer crop products (Wang et al., 2016), such as MON 88701 cotton, MON 87708 soybean, MON 87419 maize, and MON 87429 maize, which completed FDA consultation in 2013 (BNF 000135), 2011 (BNF 000125), 2016 (BNF 000148), and 2022 (BNF 000173), respectively. The N-terminal amino acid difference between MON 94313 DMO protein and previously reviewed DMO proteins does not impact the high specificity for dicamba as a substrate. Full safety assessments were also conducted and deregulation decisions were received from USDA-APHIS for both MON 88701 cotton (USDA-APHIS # 12-185-01p), MON 87708 soybean (USDA-APHIS # 10-188-01p) in 2015, and MON 87419 maize (USDA-APHIS # 15-113-01p) in 2016. MON 87429 maize is currently under review by the USDA (USDA-APHIS # 19-316-01p). The safety of DMO protein has also been favourably assessed following extensive reviews by regulatory agencies in at least 12 different countries. Although there are minor differences in amino acid sequence, given the high level of amino

acid sequence identity between them, it is expected that the DMO protein expressed in MON 94313 is identical in structure of the catalytic site, function, immunoreactivity and substrate specificity to previously reviewed DMO proteins. Therefore, all acute toxicology, digestibility and heat susceptibility studies reported on DMO proteins in BNF 000125 for MON 87708 soybean DMO and BNF 000135 for MON 88701 cotton DMO are applicable to the safety assessment of DMO protein expressed in MON 94313.

MON 94313 also contains a *pat* expression cassette that expresses the PAT protein. The safety of PAT proteins, present in numerous commercial biotechnology-derived products (e.g., A5547-127 soybean), has been extensively assessed and in 1997 a tolerance exemption was issued for PAT protein by U.S. EPA (40 CFR § 180.1151). The safety of PAT proteins has been confirmed following extensive reviews by regulatory agencies in at least 15 different countries for more than 30 biotechnology-derived events in several different crop species (e.g., maize, soybean, cotton, canola and sugarbeet). The lack of any documented reports of adverse effects of PAT-containing crops since their commercial introduction further confirms the safety of the PAT protein. The amino acid sequence of the PAT protein expressed in MON 94313 is identical to the wild type PAT protein encoded by *S. viridochromogenes* except for the first methionine, which is removed due to co-translational processing in MON 94313. N-terminal methionine cleavage is common and naturally occurs in the vast majority of proteins (Meinzel and Giglione, 2008). Thus, all acute toxicology, digestibility, and heat susceptibility studies that were part of prior safety assessments of PAT proteins are applicable to the PAT protein expressed in MON 94313.

The FT_T.1 protein in MON 94313 is nearly identical to the FT_T protein produced in MON 87429 maize which completed FDA consultation in 2022 (BNF 000173). The FT_T.1 protein in MON 94313 soybean differs from FT_T protein in MON 87429 maize by 3 amino acids. These minor changes were introduced to increase the enzymatic activity of the FT_T.1 protein for 2,4-D relative to FT_T and do not have an effect on the structure, immunoreactivity or specificity of the FT_T.1 protein compared to FT_T. Therefore, all acute toxicology, digestibility and heat susceptibility studies reported for the FT_T protein in BNF 000173 for MON 87429 maize are applicable to the safety assessment of FT_T.1 protein expressed in MON 94313.

MON 94313 also contains a *TDO* expression cassette that expresses a triketone dioxygenase protein (TDO) that is identical to the native rice protein HIS1 (Maeda et al., 2019). A multistep approach to the safety assessment of the TDO protein was conducted according to guidelines established by the Codex Alimentarius Commission (Codex Alimentarius, 2009) and OECD, which embody the principles and guidance of the FDA's 1992 policy on foods from new plant varieties. 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 animal health; 2) characterization of the physicochemical and functional properties of expressed protein; 3) quantification of the expressed protein's expression 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; 6) evaluation of the stability of the expressed protein after heat treatment; and 7) investigation of potential animal toxicity through an animal assay. The data collected to address these elements collectively supports the conclusion that dietary exposure to TDO protein derived from MON 94313 poses no meaningful risk to human or animal health.

Compositional Analysis of MON 94313 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 94313 and the conventional control grown at five sites representative of typical agricultural regions for soybean production in the United States during the 2020 field season. The compositional analysis provided a comprehensive comparative assessment of the levels of key nutrients, anti-nutrients of MON 94313 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 94313 and conventional control and supported the conclusion that MON 94313 soybean is compositionally equivalent to the conventional control. These results support the overall food and feed safety of MON 94313.

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 94313 and its progeny are as safe and nutritious as food and feed derived from conventional soybean. The food and feed safety of MON 94313 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 soybean genome. The genetic elements are present in the expected order and are inherited following Mendelian principles.
2. Extensive evaluation of the DMO, PAT, FT_T.1 and TDO proteins demonstrates that they do not pose any meaningful risk to food or feed safety.
3. The comprehensive compositional assessment demonstrated that MON 94313 grain and forage are compositionally equivalent to grain and forage from conventional soybean.

Therefore, the data herein demonstrate that the food and feed derived from MON 94313 and its progeny are as safe and nutritious as food and feed derived from conventional soybean.