# EXECUTIVE SUMMARY
# to
# Application to Food Standards Australia New Zealand for the Inclusion of Corn MON 87419 in *Standard 1.5.2 - Food Derived from Gene Technology*

Submitted by:

**Monsanto Australia Limited**

**Level 12 / 600 St Kilda Road,**

**Melbourne Victoria 3004**

**7 August 2015**

# EXECUTIVE SUMMARY

## MON 87419 Product Description

Monsanto Company has developed MON 87419 maize that is tolerant to dicamba (3,6-dichloro-2-methoxybenzoic acid) and glufosinate (2-amino-4-(hydroxymethylphosphinyl) butanoic acid) herbicides. MON 87419 contains a demethylase gene from *Stenotrophomonas maltophilia* that expresses a dicamba mono-oxygenase (DMO) protein to confer tolerance to dicamba herbicide and the phosphinothricin N-acetyltransferase (*pat*) gene from *Streptomyces viridochromogenes* that expresses the PAT protein to confer tolerance to glufosinate herbicide.

MON 87419 will offer maize growers multiple choices for effective weed management including tough to control and herbicide resistant broadleaf weeds. The combination of these two unique herbicide mechanisms-of-action provides an effective weed management system for maize production. Dicamba provides effective control of over 95 annual and biennial weed species, and suppression of over 100 perennial broadleaf and woody plant species. Glufosinate, a broad-spectrum contact herbicide, provides nonselective control of approximately 120 broadleaf and grass weeds. Additionally, dicamba and glufosinate provide control of herbicide-resistant weeds, including glyphosate-resistant biotypes of Palmer amaranth (*Amaranthus palmeri*), marestail (*Conyza canadensis*), common ragweed (*Ambrosia artemisiifolia*), giant ragweed (*Ambrosia trifida*) and waterhemp (*Amaranthus tuberculatus*).

MON 87419 will likely be combined, through traditional breeding methods, with other deregulated events (*e.g.,* glyphosate-tolerant). The in-crop use of dicamba and glufosinate herbicides, in addition to glyphosate herbicide, provides improved weed management options in maize to control a broad spectrum of grass and broadleaf weed species and effective control of weeds resistant to several herbicide families. Additionally, MON 87419 combined with glyphosate-tolerant maize systems will provide: 1) an opportunity for an efficient, effective weed management system for hard-to-control and herbicide-resistant weeds; 2) a flexible system for two additional herbicide mechanisms-of-action for in-crop application in current maize production systems as recommended by weed science experts to manage future weed resistance development; 3) an option to delay or prevent further resistance to glyphosate and other critically important maize herbicides; 4) crop safety to dicamba, glufosinate and glyphosate; and 5) additional weed management tools to enhance weed management systems necessary to maintain or improve maize yield and quality to meet the growing needs of the food, feed, and industrial markets.

## Molecular Characterisation of MON 87419 Verifies the Integrity and Stability of the Inserted DNA

MON 87419 was produced by *Agrobacterium*-mediated transformation of maize tissue using the 2T-DNA transformation plasmid vector PV-ZMHT507801. This plasmid vector contains two separate T-DNAs (transfer DNA), that are each delineated by Right and Left Border regions. The first T-DNA, designated as T-DNA I, contains the *dmo* expression cassette and the *pat* expression cassettes. The second T-DNA, designated as T-DNA II, contains the *cp4 epsps* expression cassette for selection. During transformation, both T-DNAs were inserted into the maize genome. Subsequently, traditional breeding, segregation, selection and screening were used to isolate plants that contained the *dmo* expression cassette and the

*pat* expression cassettes (T-DNA I) and did not contain the *cp4 epsps* expression cassette (T-DNA II).

Characterization of the DNA insert in MON 87419 was conducted using a combination of sequencing, PCR, and bioinformatics. The results of this characterization demonstrate that MON 87419 contains one copy of the intended transfer DNA (T-DNA I) containing the *dmo* expression cassette and *pat* expression cassettes 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 87419 by Next Generation Sequencing and Junction Sequence Analysis (NGS/JSA) demonstrated that MON 87419 contained a single intended DNA insert. These whole-genome sequence analyses provided a comprehensive assessment of MON 87419 to determine the presence and identity of sequences derived from PV-ZMHT507801 and demonstrated that MON 87419 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 87419 was used to determine the complete sequence of the single DNA insert from PV-ZMHT507801, 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-ZMHT507801 T-DNA I. Furthermore, the genomic organization at the insertion site was assessed by comparing the sequences flanking the T-DNA I insert in MON 87419 to the sequence of the insertion site in conventional maize. This analysis determined that no DNA rearrangement occurred at the insertion site in MON 87419 upon DNA integration, although a 602 bp deletion was observed.

- Generational stability analysis by NGS/JSA demonstrated that the single PV-ZMHT507801 T-DNA I insert in MON 87419 has been maintained through five breeding generations, thereby confirming the stability of the T-DNA I in MON 87419.

- Segregation analysis corroborates the insert stability demonstrated by NGS/JSA and independently establishes the nature of the T-DNA I as a single chromosomal locus.

Taken together, the characterization of the genetic modification in MON 87419 demonstrates that a single copy of the intended T-DNA I was stably integrated at a single locus of the maize genome and that no plasmid backbone sequences are present in MON 87419.

**DMO and PAT are safe for consumption in food or feed**

MON 87419 contains a *dmo* expression cassette that encodes for a single MON 87419 DMO precursor protein that is post-translationally processed into two forms of the DMO protein; referred to as MON 87419 DMO+12 and MON 87419 DMO+7. MON 87419 DMO+7 is identical to MON 87419 DMO+12 with the exception that it does not contain the first five amino acids of MON 87419 DMO+12. Therefore, MON 87419 DMO protein will be used to

refer to both forms of the protein collectively and distinctions will only be made where necessary.

DMO proteins produced in MON 87419 are also present in MON 88701 cotton and MON 87708 soybean, which completed FDA consultation in 2013 (BNF 000135) and 2011 (BNF 000125), respectively. Full safety assessments were also conducted and deregulation decisions were received from USDA-APHIS for both MON 88701 (USDA-APHIS # 12-185-01p) and MON 87708 (USDA-APHIS # 10-188-01p) in 2015. Data, demonstrating the safety of DMO, were satisfactorily reviewed by U.S. agencies in accordance with the review responsibilities under the Coordinated Framework, resulting in full authorization of these products in the U.S. The safety of DMO protein has been favorably assessed following extensive reviews by regulatory agencies in at least eight different countries. Although there are minor differences in amino acid sequence, the DMO proteins expressed in MON 87419 are identical in structure of the catalytic site, function, immunoreactivity and specificity to previously reviewed DMO proteins. Therefore, all acute toxicology, digestibility and heat susceptibility studies reported on DMO proteins in BNF 000125 and BNF 000135 are applicable to the safety assessment of MON 87419.

MON 87419 also contains a *pat* expression cassette that encodes for a phosphinothricin N-acetyltransferase protein herein referred to as PAT.

The safety of PAT protein, present in numerous commercial biotechnology-derived crops, has been extensively assessed and in 1997 a tolerance exemption was issued for PAT proteins regardless of the encoding gene or crop by U.S. EPA (U.S. EPA, 1997). The safety of PAT protein has been favorably assessed following extensive reviews by regulatory agencies in at least 11 different countries for more than 38 biotechnology-derived events in eight different species. The lack of any documented reports of adverse effects of PAT-containing crops since their introduction further confirms the safety of the PAT protein. The amino acid sequence of the PAT protein expressed in MON 87419 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 87419. N-terminal methionine cleavage is common and naturally occurs in the vast majority of proteins (Meinnel and Giglione, 2008). Thus, prior safety assessments of PAT protein are directly applicable to the PAT protein expressed in MON 87419.

In addition, the donor organisms for the DMO and PAT protein coding sequences, *S. maltophilia* and *S. viridochromogenes*, respectively, are ubiquitous in the environment and are not known or reported to pose a risk of human allergenicity or pathogenicity to humans or animals. Bioinformatics analysis determined that the DMO and PAT proteins lack structural similarity to known allergens, gliadins, glutenins, or protein toxins and acute toxicology studies demonstrated that the DMO and PAT proteins have no acute oral toxicity in mice at the highest levels tested. The DMO and PAT proteins are rapidly digested by proteases found in the human gastrointestinal tract (pepsin and pancreatin).

Finally, based on results from the acute toxicity assays and levels of DMO and PAT in MON 87419 grain, margins of exposure for acute dietary intakes of DMO and PAT are 810,000 and 620,000, respectively, for the general population and 350,000 and 270,000, respectively, for children one-two years of age. Taken together, these data support a conclusion that DMO and PAT from MON 87419 or its progeny pose no meaningful risk to human and animal health.

**Compositional Analysis of MON 87419 Demonstrates Equivalence to the Conventional Crop**

Compositional analysis was conducted on grain and forage of MON 87419 treated with dicamba and glufosinate and a conventional control grown at five sites in the United States during 2013. The evaluation of MON 87419 followed considerations relevant to the compositional quality of maize as defined by the OECD consensus document (OECD, 2002a). Grain samples were analyzed for levels of nutrients including proximates (protein, fat, ash, moisture), amino acids (18 components), fatty acids (22 components), carbohydrates by calculation, acid detergent fiber (ADF), neutral detergent fiber (NDF), total dietary fiber (TDF), minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc), and vitamins [A (β-carotene), B1, B2, B6, E (α-tocopherol), niacin, and folic acid]. The anti-nutrients analyzed in grain were phytic acid and raffinose. Secondary metabolites analyzed in grain were furfural, ferulic acid, and p-coumaric acid. Forage samples were analyzed for levels of proximates, carbohydrates by calculation, fiber (ADF, NDF), and minerals (calcium, and phosphorus). In all, 78 different components were analyzed.

Of the 78 measured compositional components, copper, furfural, and 13 fatty acids (caprylic, capric, lauric, myristic, myristoleic, pentadecanoic, pentadecenoic, heptadecanoic, heptadecenoic, gamma linolenic, eicosadienoic, eicosatrienoic, and arachidonic acids) had more than 50% of the observations below the assay limit of quantitation (LOQ) and were excluded from the statistical analyses. Moisture values for grain and forage were measured for conversion of components from fresh to dry weight, but were not statistically analyzed. Therefore, 61 components were statistically analyzed (53 in grain and eight in forage). The statistical comparison of MON 87419 and the conventional control was based on compositional data combined across all field sites. Statistically significant differences were identified at the 5% level ($\alpha = 0.05$).

Of the 61 components statistically assessed, 60 showed no significant differences between MON 87419 and the conventional control. One component (manganese in grain) showed a significant difference between MON 87419 and the conventional control. For this one component, the mean difference in the component values between MON 87419 and the conventional control was less than the range value of the conventional control. The MON 87419 mean component value was also within the range of manganese values observed in the literature and the ILSI-CCDB. These data indicated that the statistically significant difference observed for manganese was not compositionally meaningful from a food and feed safety or nutritional perspective.

These results support the overall conclusion that MON 87419 was not a major contributor to variation in component levels in maize grain and forage and confirmed the compositional equivalence of MON 87419 to the conventional control in levels of these components.

**Conclusion**

The data and information presented in this safety summary, supported by previous conclusions of food and feed safety for DMO-expressing products (A1063 and A1080) and current tolerance exemptions for PAT proteins (U.S. EPA, 1997), along with the detailed compositional analysis, demonstrate that the food and feed derived from MON 87419 and its progeny are as safe and nutritious as food and feed derived from conventional maize. The food/feed safety of MON 87419 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 maize genome. The genetic elements are present in the expected order and are inherited following Mendelian principles.

2. The DMO and PAT proteins have previously been evaluated across various crop species and found to not pose meaningful risk to food or feed safety.

3. Based on bioinformatics searches of the T-DNA I 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 I insertion in MON 87419.

4. The comprehensive compositional assessment demonstrated that MON 87419 grain and forage are compositionally equivalent to grain and forage from conventional maize.

Therefore, the consumption of MON 87419 and its progeny, and the food and feed derived from it will be in compliance with all applicable requirements of the FSANZ and the data herein demonstrate that the food and feed derived from MON 87419 and its progeny are as safe and nutritious as food and feed derived from conventional maize.