EXECUTIVE SUMMARY
to
Application to Food Standards Australia New Zealand for the Inclusion of Corn MON 87429 in *Standard 1.5.2 - Food Derived from Gene Technology*

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Food/Feed Safety and Nutritional Assessment of MON 87429

Monsanto Company has developed herbicide tolerant MON 87429 maize, which is tolerant to the herbicides dicamba, glufosinate, aryloxyphenoxypropionate (AOPP) acetyl coenzyme A carboxylase (ACCase) inhibitors (so called "FOPs" herbicides such as quizalofop) and 2,4-dichlorophenoxyacetic acid (2,4-D). In addition, it provides tissue-specific glyphosate tolerance to facilitate the production of viable hybrid maize seeds. MON 87429 contains a demethylase gene from *Stenotrophomonas maltophilia* that expresses a dicamba mono-oxygenase (DMO) protein to confer tolerance to dicamba herbicide, the phosphinothricin-N-acetyltransferase (*pat*) gene from *Streptomyces viridochromogenes* that expresses the PAT protein to confer tolerance to glufosinate herbicide and the *ft_t* 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) that confers tolerance to FOPs and 2,4-D herbicides. MON 87429 maize also produces the 5-enolpyruvylshikimate-3-phosphate synthase protein from *Agrobacterium* sp. strain CP4 (CP4 EPSPS). MON 87429 maize utilizes an endogenous maize regulatory element to target CP4 EPSPS mRNA for degradation in tassel tissues, resulting in reduced CP4 EPSPS protein expression in pollen. Appropriately timed glyphosate applications produce a non-viable pollen phenotype and allow for desirable cross pollinations to be made in maize without using mechanical or manual detasseling methods to control self-pollination in female inbred parents.

MON 87429 maize 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 mechanisms-of-action provides an effective weed management system for maize production. Dicamba provides effective control of over 95 annual and biennial broadleaf weed species, and suppression of over 100 perennial broadleaf and woody plant species. Glufosinate, a broad-spectrum contact herbicide, provides effective control of approximately 70 broadleaf and 60 grass weeds. Quizalofop, a selective postemergence herbicide, provides effective control of approximately 35 annual and perennial grass weeds including glyphosate-resistant grasses. 2,4-D provides effective control of over 70 annual and biennial broadleaf weed species, and suppression of over 30 perennial broadleaf species. Additionally, dicamba, glufosinate, and 2,4-D individually or in certain combinations 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 87429 maize will likely be combined, through traditional breeding methods, with other deregulated events (e.g., glyphosate-tolerant). MON 87429 maize combined with glyphosate-tolerant maize 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 mechanisms-of-action for in-crop application in current maize production systems; 3) an opportunity to delay selection for further resistance to glyphosate and other herbicides that are important in crop production; 4) excellent crop tolerance to dicamba, glufosinate, quizalofop, 2,4-D 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.

Tissue-specific expression of CP4 EPSPS protein in MON 87429, allowing for glyphosate induced non-viable pollen phenotype, is the second generation of Monsanto's Roundup® Hybridization System (RHS) for hybrid seed production. The first-generation RHS event, MON 87427 maize, was approved by FSANZ in 2012 (A1066). The second-generation RHS trait in MON 87429 allows inbred MON 87429 lines, treated with glyphosate at the appropriate timings, to serve as a female parent in the production of hybrid seed. Inbred MON 87429 lines receive two glyphosate applications at vegetative growth stages ranging from V8 to V13, resulting in the intended non-viable pollen phenotype due to tissue-specific glyphosate susceptibility. In hybrid maize production systems, these inbred MON 87429 plants sprayed with glyphosate during tassel development will be pollinated by pollen donor plants that contain a glyphosate tolerance trait (e.g. NK603). Donor inbred plants are cultivated in proximity to inbred MON 87429 plants and the donor inbred plants are not impacted by RHS required glyphosate applications, due to full glyphosate tolerance in all tissues. This cross results in hybrid offspring (MON 87429 × NK603) with full tolerance to glyphosate, both in vegetative and reproductive tissues, as well as tolerance to dicamba, glufosinate, quizalofop, and 2,4-D herbicides. For weed control in hybrid seed production fields, glyphosate may be applied to MON 87429 maize at vegetative stages as directed on glyphosate agricultural product labels, at the same rates used in previously deregulated Roundup Ready® maize events.

The RHS trait in MON 87429 maize offers the same benefits to hybrid maize seed production as the RHS trait in MON 87427, described in detail in A1066. Briefly, these benefits include enabling hybrid seed producers to discontinue the practice of manually or mechanically detasseling female inbred plants in their production field, which must occur during a critical 3-4 day time period of maize tassel development, and can be influenced by changes in weather (e.g., extreme heat). The ability to spray MON 87429 maize inbreds with glyphosate (between V8 to V13), in place of detasseling, provides flexibility to hybrid maize seed producers and reduces the cost of hybrid seed production by removing the reliance on costly, labor intensive manual/mechanical detasseling. An additional benefit of including the RHS trait in MON 87429 maize, along with dicamba-, glufosinate-, and quizalofop and 2,4-D-tolerance traits, is the reduction in the number of trait loci that need to be combined, via traditional breeding methods, to generate new, RHS containing stack product offerings.

Molecular Characterisation of MON 87429 Verifies the Integrity and Stability of the Inserted DNA

MON 87429 was produced by *Agrobacterium* mediated-transformation of maize tissue using the T-DNA transformation vector PV-ZMHT519224. This plasmid vector contains a single transfer DNA (T-DNA), that is delineated by Right and Left Border regions. The T-DNA contains the *pat*, *dmo, ft_t*, and *cp4 epsps* expression cassettes. Following transformation, traditional breeding, segregation, selection and screening were used to isolate those plants that contain the *pat, dmo*, *ft_t,* and *cp4 epsps* expression cassettes and do not contain the backbone sequences.

Characterization of the DNA insert in MON 87429 was conducted using a combination of sequencing, polymerase chain reaction (PCR), and bioinformatics. The results of this characterization demonstrate that MON 87429 contains one copy of the intended T-DNA

containing the *pat*, *dmo*, *ft_t*, and *cp4 epsps* expression cassettes that is stably inherited over multiple generations and segregates according to Mendelian principles.

PAT, DMO, FT_T and CP4 EPSPS are safe for consumption in food or feed

MON 87429 contains a *dmo* expression cassette that expresses a single MON 87429 DMO precursor protein that is post-translationally processed during the chloroplast targeting process into two forms of the DMO protein; referred to as MON 87429 DMO+1 and MON 87429 DMO+0. MON 87429 DMO+1 is identical to MON 87429 DMO+0 with the exception that it contains an additional amino acid on the N-terminus, a cysteine residue, derived from the alternative processing of the chloroplast transit peptide APG6. Given this degree of similarity, MON 87429 DMO protein will be used to refer to both forms of the protein collectively and distinctions will only be made where necessary. DMO proteins highly similar to those produced in MON 87429 are also present in MON 88701 cotton, MON 87708 soybean and MON 87419 maize (Wang *et al*., 2016), which had been approved by FSANZ in 2014 (A1080), 2012 (A1063) and 2016 (A1118), respectively. The N-terminal amino acid difference between MON 87429 DMO proteins and previously reviewed DMO proteins does not impact the high specificity for dicamba as a substrate. Data, demonstrating the safety of DMO, were also 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 favourably assessed following extensive reviews by regulatory agencies in at least 12 different countries. Although there are minor differences in amino acid sequence, the DMO proteins expressed in MON 87429 are identical in structure of the catalytic site, function, immunoreactivity and substrate specificity to previously reviewed DMO proteins. Thus, prior safety assessments of DMO proteins are applicable to the safety assessment of DMO protein expressed in MON 87429.

MON 87429 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. T25, TC1507, A5547-127 and DAS-59122-7), 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 87429 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 87429. 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 proteins are applicable to the PAT protein expressed in MON 87429.

The CP4 EPSPS protein in MON 87429 has the same sequence as the CP4 EPSPS proteins produced in several other commercially available products that have been reviewed by the FSANZ (e.g. MON 87427 A1066 in 2012 and MON 88017 A548 in 2006). The safety and mode-of-action of CP4 EPSPS proteins is well documented and is the subject of numerous publications (Harrison *et al*., 1996; Hoff *et al*., 2007; ILSI-CERA, 2010; U.S. EPA, 1996). Additionally, in 1996 the U.S. EPA established an exemption from the requirement of a tolerance for residues of the plant pesticide inert ingredient CP4 EPSPS and the genetic

material necessary for its production in all plants (40 CFR § 174.523, redesignated from § 180.1174, effective April 25, 2007). The safety of the CP4 EPSPS protein as expressed in MON 88017 and MON 87427 maize has also been reviewed and approved in at least 12 countries. Thus, prior safety assessments of CP4 EPSPS protein are applicable to the CP4 EPSPS protein expressed in MON 87429.

MON 87429 also contains an *ft_t* expression cassette that expresses a FOPs and 2,4-D dioxygenase protein (FT_T) that is the modified version of the R-2,4-dichlorophenoxypropionate dioxygenase (RdpA) protein (Müller *et al*., 2006). A multistep approach to the safety assessment of the FT_T 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 proteins' 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 FT_T protein derived from MON 87429 poses no meaningful risk to human or animal health.

Compositional Analysis of MON 87419 Demonstrates Equivalence to the Conventional Crop

Compositional analysis was conducted on grain and forage of MON 87429 and a conventional control grown at five sites in the United States during 2017. The evaluation of MON 87429 focused on key nutrients and antinutrients of maize grain and forage as based on the OECD consensus document on compositional considerations. Harvested grain samples were assessed for moisture and levels of nutrients including proximates (protein, total fat and ash), essential amino acids (10 components), linoleic acid (essential fatty acid), carbohydrates by calculation, and fiber (ADF and NDF). Grain samples were also assessed for levels of antinutrients (phytic acid and raffinose). Harvested forage samples were assessed for moisture and levels of nutrients including proximates (protein, total fat and ash), carbohydrates by calculation and fiber (ADF and NDF). In all, 27 different components were analysed in forage and grain. Moisture values for grain and forage were measured for conversion of components to dry weight, but were not statistically analysed. Therefore, 25 components were statistically analysed for all samples.

There were no statistically significant differences ($p<0.05$) for 23 of the 25 components analysed. There were two components (total fat and linoleic acid in grain) that showed a statistically significant difference ($p<0.05$) between MON 87429 and the conventional control. For these components, the mean difference between MON 87429 and the conventional control was less than the conventional control range values and the MON 87429 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 differences observed were not biologically meaningful from a food and feed safety perspective. These

results support the conclusion that MON 87429 maize is compositionally equivalent to the conventional control in levels of key nutrients and anti-nutrients in grain and forage.

Conclusion

The data and information presented in this safety summary demonstrate that the food and feed derived from MON 87429 and its progeny are as safe and nutritious as food and feed derived from conventional maize. The food/feed safety of MON 87429 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 MON 87429 genome and the absence of plasmid backbone DNA. The genetic elements are present in the expected order and are inherited following Mendelian principles.

2. Extensive evaluation of the DMO, PAT, CP4 EPSPS and FT_T proteins demonstrates that they do not pose any meaningful risk to food or feed safety.

3. MON 87429 maize utilizes an endogenous maize regulatory element to target CP4 EPSPS mRNA for degradation in tassel tissues, resulting in reduced CP4 EPSPS protein expression in pollen with no impact on endogenous plant gene expression.

4. The comprehensive compositional assessment demonstrated that MON 87429 grain and forage is compositionally equivalent to grain and forage from conventional maize.

The data herein demonstrate that the food and feed derived from MON 87429 and its progeny are as safe and nutritious as food and feed derived from conventional maize.