EXECUTIVE SUMMARY
to
Application to Food Standards Australia New Zealand for the Inclusion of Corn MON 87411 in *Standard 1.5.2 - Food Derived from Gene Technology*

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EXECUTIVE SUMMARY

MON 87411 Product Description

Monsanto Company has developed biotechnology-derived maize, MON 87411, that confers protection against corn rootworm (CRW) (*Diabrotica spp*.) and tolerance to the herbicide glyphosate. MON 87411 contains a suppression cassette that expresses an inverted repeat sequence designed to match the sequence of western corn rootworm (WCR; *Diabrotica virgifera virgifera*). The expression of the suppression cassette results in the formation of a double-stranded RNA (dsRNA) transcript containing a 240 bp fragment of the WCR *Snf7* gene (DvSnf7). Upon consumption, the plant-produced dsRNA in MON 87411 is recognized by the CRW's RNA interference (RNAi) machinery resulting in down-regulation of the targeted DvSnf7 gene leading to CRW mortality. MON 87411 also contains a *cry3Bb1* gene that produces a modified *Bacillus thuringiensis* (subsp. *kumamotoensis*) Cry3Bb1 protein to protect against CRW larval feeding. In addition, MON 87411 contains the *cp4 epsps* gene from *Agrobacterium* sp. strain CP4 that encodes for the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) protein, which confers tolerance to glyphosate, the active ingredient in Roundup® agricultural herbicides.

MON 87411 builds upon the current *Bt* protein-based mode-of-action (MOA) for CRW control by the addition of a new RNA-mediated MOA that offers enhanced control of target insect pests and prolonged durability of existing *Bt* technologies designed to control CRW. MON 87411 will provide benefits to growers similar to those obtained by use of existing CRW-protected maize hybrids, which include reduced need for insecticides and associated improvements in worker safety, increased yield protection, and water conservation. MON 87411 is also glyphosate tolerant and will continue to provide benefits associated with conservation tillage methods, including reduced soil erosion, reduced fuel and labor costs, improved air quality and conservation of soil moisture.

MON 87411 will not be offered for commercial use as a stand-alone product, but will be combined, through traditional breeding methods, with other deregulated biotechnology-derived traits to provide protection against both above-ground and below-ground maize pests as well as tolerance to multiple herbicides. These next generation combined-trait maize products will offer broader grower choice, improved production efficiency, increased pest control durability, and enhanced grower profit potentials.

History of Use of the Host and Donor Organisms

MON 87411 contains a suppression cassette that expresses an inverted repeat sequence designed to match the sequence of western corn rootworm (WCR; *Diabrotica virgifera virgifera*). MON 87411 also contains a cry3Bb1 coding sequence that produces a modified *Bacillus thuringiensis* (subsp. *kumamotoensis*) Cry3Bb1 protein to protect against CRW larval feeding. In addition, MON 87411 contains the cp4 epsps coding sequence from *Agrobacterium* sp. strain CP4 that encodes for the 5-enolpyruvylshikimate-3-phosphate

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synthase (EPSPS) protein, which confers tolerance to glyphosate, the active ingredient in Roundup agricultural herbicides.

Maize is widely used for a variety of food and feed purposes, and it is intended that MON 87411 will be utilized in the same manner and for the same uses as conventional maize. Maize grain and its processed products are consumed in a multitude of human food and animal feed products. Maize forage (as silage) is extensively consumed as an animal feed by ruminants.

Nature of the Genetic Modification

MON 87411 was developed through *Agrobacterium*-mediated transformation of maize immature embryos from line LH244 utilizing plasmid vector PV-ZMIR10871. PV-ZMIR10871 contains one transfer DNA (T-DNA) that is delineated by Left and Right Border regions. The T-DNA contains the DvSnf7 suppression cassette, the *cry3Bb1* expression cassette, and the *cp4 epsps* expression cassette. The DvSnf7 suppression cassette is regulated by the *e35S* promoter from the 35S RNA of cauliflower mosaic virus (CaMV), the heat shock protein 70 (*Hsp70*) intron from *Zea mays*, and the 3' untranslated sequence of the *E9* gene from *Pisum sativum*. The *cry3Bb1* expression cassette is regulated by the *pIIG* promoter from *Zea mays*, the chlorophyll a/b binding protein (CAB) leader from *Triticum aestivum*, the *Ract1* intron from *Oryza sativa*, and the heat shock protein 17 (*Hsp17*) 3′ untranslated region from *Triticum aestivum*. The *cp4 epsps* expression cassette is regulated by the *TubA* promoter from *Oryza sativa*, the *TubA* leader from *Oryza sativa*, the *TubA* intron from *Oryza sativa*, the *CTP2* chloroplast-targeting sequence from *Arabidopsis thaliana*, and the *TubA* 3' untranslated region from *Oryza sativa*.

Characterisation of the DNA insert in MON 87411 was conducted using a combination of sequencing, polymerase chain reaction (PCR), and bioinformatics. The results of this characterisation demonstrate that MON 87411 contains one copy of the intended T-DNA containing the DvSnf7 suppression cassette and the *cry3Bb1* and *cp4 epsps* expression cassettes that is stably integrated at a single locus and is inherited according to Mendelian principles over multiple generations.

Characterisation of Novel Proteins or Other Novel Substances

MON 87411 expresses the Cry3Bb1 protein and DvSnf7 RNA to control coleopteran corn rootworm (*Diabrotica* spp.) pests and the CP4 EPSPS protein to provide tolerance to glyphosate herbicides in maize.

A multistep approach was used to characterise and assess the safety of the CP4 EPSPS and Cry3Bb1 proteins expressed in MON 87411. The expression levels of the CP4 EPSPS and Cry3Bb1 proteins in selected tissues of MON 87411 were determined and exposure to humans and animals through diet was evaluated. In addition, the donor organisms for the CP4 EPSPS and Cry3Bb1 protein coding sequences, *Agrobacterium* sp. strain CP4 and *Bacillus thuringiensis* ssp *kumamotoensis*, are ubiquitous in the environment and are not commonly known for human or animal pathogenicity or allergenicity. Bioinformatics analysis determined that the CP4 EPSPS and Cry3Bb1 proteins lack structural similarity to known allergens or protein toxins. As has been previously shown in safety assessments of

other Roundup Ready and Cry3Bb1-containing crops, the CP4 EPSPS and Cry3Bb1 proteins are rapidly digested in simulated digestive fluids and demonstrate no acute oral toxicity in mice at the levels tested. Hence, the consumption of the CP4 EPSPS and Cry3Bb1 proteins from MON 87411 or its progeny poses no meaningful risk to human and animal health or an increased plant pest risk.

DvSnf7 RNA from MON 87411 is a dsRNA that upon consumption by western corn rootworm causes gene suppression of the targeted DvSnf7 gene. Based on the ubiquitous nature of RNAi suppression utilizing dsRNA in a wide variety of consumed plant species, demonstration of the specificity of DvSnf7 suppression in CRW, the long history of safe consumption of RNA from a range of sources, and the apparent lack of toxicity or allergenicity of dietary RNA; the DvSnf7 RNAi suppression sequence used in MON 87411 poses no observed or theoretical risks to humans or animals. Therefore, the consumption of the DvSnf7 RNA from MON 87411 or its progeny is considered safe for humans and animals and poses no increased plant pest risk.

Cry3Bb1, CP4 EPSPS and DvSnf7 RNA are Safe for Consumption in Food and Feed

Both Cry3Bb1 and CP4 EPSPS proteins produced in MON 87411 are also present in MON 88017 maize, which received FSANZ approval under Application A548 in 2006. MON 88017 and data demonstrating its safety were also satisfactorily reviewed by U.S. agencies in accordance with the review responsibilities under the Coordinated Framework, resulting in full approval of the product in the U.S. Monsanto also gained approval from FSANZ for MON 863 (A484) in 2003 that contains a similar Cry3Bb1 protein. Full safety assessments were also conducted on MON 863 resulting in USDA deregulation and U.S. EPA registration. The safety of these proteins as expressed in MON 88017 and MON 863 has also been reviewed and approved in numerous other countries (*e.g.*, Argentina, Canada, China, the European Union, Japan, Korea, Mexico, Philippines, Taiwan).

The Cry3Bb1 protein belongs to a family of Cry proteins from *B. thuringiensis* that has been used commercially in the U.S. to produce microbial-derived products with insecticidal activity. There are at least 180 registered microbial *B. thuringiensis* products and over 120 microbial products in the European Union. Applications of sporulated *B. thuringiensis* have a long history of safe use for pest control in agriculture, especially in organic farming. The consumption of agricultural food crops sprayed with commercial *B. thuringiensis* microbial pesticides has a 50-year history of safe use and crops expressing proteins derived from *B. thuringiensis* have been commercially cultivated since 1996.

The Cry3Bb1 protein present in MON 87411 has over 99% amino acid identity to the Cry3Bb1 protein produced in MON 863 and the deduced amino acid sequence is identical to that produced from the expression cassette present in MON 88017. MON 863 and MON 88017 have been grown collectively on tens of millions of acres in the U.S. since their introductions. On March 31, 2004, U.S. EPA established an exemption from the requirement of a tolerance for residues of the plant-incorporated protectant Cry3Bb1 in maize (40 CFR § 174.518, revised and redesignated from § 180.1214, effective July 24, 2007). U.S. EPA

also completed safety reviews of Cry3Bb1 in 2010 for its Biopesticide Registration Action Document for MON 863 (originally registered February 24, 2003) and MON 88017 (originally registered December 15, 2005).

The 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS: EC 2.5.1.19) family of enzymes is ubiquitous in plants and micro-organisms and their properties have been well studied. Bacterial and plant EPSPS enzymes are mono-functional with molecular mass of 44-48 kD. EPSPS is a key enzyme involved in aromatic amino acid biosynthesis and catalyzes the reaction where the enolpyruvoyl group from phosphoenol pyruvate (PEP) is transferred to the 5-hydroxyl of shikimate-3-phosphate to form 5-enolpyruvylshikimate-3-phosphate (EPSP) and inorganic phosphate. The CP4 EPSPS protein expressed in MON 87411 is functionally equivalent to endogenous plant EPSPS enzymes except it displays a much reduced affinity for glyphosate. Shikimic acid is a substrate for the biosynthesis of the aromatic amino acids phenylalanine, tryptophan and tyrosine, as well as many secondary metabolites, such as tetrahydrofolate, ubiquinone, and vitamin K and the shikimic acid pathway is absent in mammals, fish, birds, reptiles and insects. CP4 EPSPS degrades rapidly in simulate gastric and intestinal fluids and no adverse effects were noted on mice gavaged with a high dose (572 mg/kg bodyweight) of this protein. Additionally, CP4 EPSPS does not have significant sequence or structural homology to known toxins or allergens. Regulatory agencies in a number of countries outside the U.S. (*e.g.*, Canada, Mexico, Australia, Japan, Brazil, Argentina) have assessed and verified the food, feed and environmental safety of CP4 EPSPS protein.

The CP4 EPSPS protein in MON 87411 is also the same as the one produced in several other commercially available crops that have been approved by FSANZ, reviewed by the FDA and deregulated by the USDA (*e.g.*, Roundup Ready varieties of soybean, maize, cotton, sugarbeet, canola, and alfalfa). The safety and mode-of-action of CP4 EPSPS proteins is well documented and is the subject of numerous publications. 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 consuming nucleic acids in food was considered by an ILSI (International Life Sciences Institute) expert panel in 2000. While DNA was the primary focus of the review, many of the same or similar conclusions can be made for RNA which contains the same basic nucleotide building blocks, with the notable exception of the replacement of thymine with uracil in RNA. Some of the major conclusions from that workshop included the following:

- Taking into account the natural variations of DNA sequences, the present use of recombinant techniques in the food chain does not introduce changes in the chemical characteristics of the DNA;
- There is no difference in the susceptibility of recDNA and other DNA to degradation by chemical or enzymatic hydrolysis;
- The metabolic fate of DNA digestion products is not influenced by the origin of the DNA;

- DNA is not toxic at levels usually ingested. Where there is potential for adverse effects, *e.g.*, gout, this is due to excessive intake, not the origin of the DNA;
- There is no indication that ingested DNA has allergenic or other immunogenic properties that would be of relevance for consumption of foods derived from GMOs;
- Uptake, integration and expression of any residual extracellular DNA fragments from foods by microorganisms of the gastrointestinal tract cannot be excluded. However, each of these circumstances is a rare event and would have to happen sequentially."

Expression of the DvSnf7 suppression cassette in MON 87411 results in the formation of a double stranded RNA transcript containing a 240 bp fragment of the WCR *Snf7* gene. Double-stranded RNAs are commonly used by eukaryotes, including plants, for endogenous gene suppression and are not known to pose novel risks from a food/feed and environment perspective. There is a history of safe consumption of the RNA molecules mediating gene suppression in plants, including those with homology to genes in humans and other animals. FSANZ noted that there is no need for additional endpoints addressing potential toxicity because there is no evidence that ingested RNA can elicit toxicity in humans regardless of sequence. In 2001, U.S. EPA established an exemption from the requirement of a tolerance for residues of nucleic acids that are part of a plant-incorporated protectant (40 CFR 174.507, redesignated from § 174.475, effective April 25, 2007).

Compositional Analyses of the GM Food

Compositional analysis was conducted on grain and forage of MON 87411, a conventional control, and 20 different commercial reference hybrids grown at eight representative sites in a 2011/2012 field production in Argentina. Production in the U.S. corn belt and Argentina maize-growing regions occur at relatively similar latitudes with an approximate 6 month offset. The average growing season temperatures and precipitation are comparable and as a result, maize hybrids developed in the U.S. are often used directly by farmers in the southern growing regions of Argentina. As such, compositional analyses from maize grown in Argentina are appropriate for a comparative safety assessment and study results are relevant to the use of this maize grown in the U.S.

The compositional analysis, based on the OECD consensus document for maize, included measurement of nutrients, anti-nutrients and secondary metabolites in conventional commercial reference hybrids to provide data on the natural variability of each compositional component analyzed. A total of 78 components were assayed (nine in forage and 69 in grain). Of the 78 components assayed, 18 had more than 50% of observations that were below the assay limit of quantitation and were therefore excluded from statistical analysis. Of the 60 remaining components statistically assessed, twelve (12) components (protein, histidine, tyrosine, oleic acid, neutral detergent fiber, copper, iron, manganese, zinc, niacin, vitamin B1 in grain, and ash in forage) showed a statistically significant difference between MON 87411 and the conventional control. For these 12 components, the mean difference in component values between MON 87411 and the conventional control, however, was less than the natural variation found within the conventional control and reference hybrid values. Additionally, MON 87411 mean component values were within the tolerance intervals of the reference hybrids, the values for maize observed in the literature, and/or the International Life Sciences Institute Crop Composition Database (ILSI-CCDB) values. These data indicated that the compositional components with statistically significant differences were not meaningful from a food and feed safety or nutritional perspective.

These results support the overall conclusion that MON 87411 was not a major contributor to variation in component levels in maize grain and forage, and confirmed the compositional equivalence of grain and forage from MON 87411 to conventional maize. These results support the overall food and feed safety of MON 87411.

Conclusion

The data and information presented in this safety summary, supported by current tolerance exemptions and approval for use for Cry3Bb1, nucleic acids, and CP4 EPSPS, demonstrate that the food and feed derived from MON 87411 and its progeny are as safe and nutritious as food and feed derived from conventional maize.