EXECUTIVE SUMMARY
to
Application to Food Standards Australia New Zealand for the Inclusion of Soybean MON 87708 in Standard 1.5.2 - Food Derived from Gene Technology

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Monsanto Company has developed biotechnology-derived soybean MON 87708 that is tolerant to dicamba herbicide. Tolerance of MON 87708 to dicamba will facilitate both preemergence and postemergence in-crop dicamba applications through the early reproductive (R1) growth stage. Dicamba provides effective control of over 95 annual and biennial weed species, and suppression of over 100 perennial broadleaf and woody plant species. Additionally, dicamba provides effective control of broadleaf weeds that are resistant to glyphosate and other commonly used herbicides, such as those in the sulfonylurea and triazine herbicide families. MON 87708 will be combined with glyphosate-tolerant soybean MON 89788 (Roundup Ready 2 Yield® soybean) utilizing traditional breeding techniques. The potential in-crop use of dicamba herbicide, in addition to glyphosate herbicide, enables an improved integrated weed management program to control a broad spectrum of grass and broadleaf weed species and convenient control of weeds resistant to several herbicide families.

MON 87708 soybean contains a gene derived from *Stenotrophomonas maltophilia* that expresses a mono-oxygenase that rapidly demethylates dicamba to an inactive metabolite 3,6-dichlorosalicylic acid (DCSA), a well known metabolite of dicamba in soybean and livestock. The existing 10 ppm pesticide residue tolerance for soybean seed in the United States and Canada supporting the current uses of dicamba on soybean is for the combined residues of parent dicamba and its metabolites, DCSA and 5-hydroxy dicamba. Studies have shown that the proposed use of dicamba on MON 87708 soybean results in total residue concentrations of parent dicamba and its metabolites (less than 0.07 ppm average residue, less than 0.5 ppm maximum residue) are well below the current 10 ppm tolerance.

The data and information presented in this safety summary demonstrate that the food and feed derived from MON 87708 are as safe and nutritious as those derived from commercially-available, conventional soybean for which there is an established history of safe consumption. This safety assessment was conducted utilizing established methods for the evaluation of biotechnology-derived products as articulated in guidelines from the Codex Alimentarius Commission and the Organization for Economic Co-operation and Development (OECD).

Safety of the Donor Organism

The *dmo* gene is derived from the bacterium *Stenotrophomonas maltophilia*. *S. maltophilia* is ubiquitous in the environment, is associated with the rhizosphere of plants, and can be found in a variety of foods and feeds. Exposure to *S. maltophilia* is incidental to its presence in food such as "ready to eat" salads, vegetables, frozen fish, milk, and poultry. *S. maltophilia* can be found in healthy individuals without causing any harm to human health, and infections in humans caused by *S. maltophilia* are extremely uncommon. Strains have been found in the transient flora of hospitalized patients as a commensal organism and, similar to the indigenous bacteria of the gastrointestinal tract, as an opportunistic pathogen. As such, *S. maltophilia* is of low virulence in immuno-compromised patients where a series of factors must occur for colonization by *S. maltophilia* in humans. The ubiquitous presence of *S. maltophilia* in the environment, the presence in healthy individuals, and the incidental presence in foods without any adverse safety reports establishes the safety of the donor organism.

Molecular Characterization of MON 87708 Verifies the Integrity and Stability of the Inserted DNA

MON 87708 was developed through *Agrobacterium tumefaciens*-mediated transformation of conventional soybean A3525 meristem tissue with the 2T-DNA plasmid vector PV-GMHT4355. PV-GMHT4355 contains two separate T-DNAs that are each delineated by Left and Right Border regions. The first T-DNA, designated as T-DNA I, contains the *dmo* expression cassette regulated by the peanut chlorotic streak caulimovirus (*PC1SV*) promoter and the pea *E9* 3′ non-translated region. The second T-DNA, designated as T-DNA II, contains the *cp4 epsps* expression cassette under the regulation of the figwort mosaic virus (*FMV*) promoter and the pea *E9* 3′ non-translated region. During transformation, both T-DNAs were inserted into the soybean genome, where T-DNA II, containing the *cp4 epsps* expression cassette, functioned as a marker gene for the selection of transformed plantlets. Subsequently, self-pollination and segregation were used to isolate a plant containing the *dmo* expression cassette but not containing the *cp4 epsps* expression cassette, resulting in the production of marker-free, dicamba-tolerant soybean MON 87708.

Molecular characterization by Southern blot analyses determined that MON 87708 contains one copy of the T-DNA I at a single integration locus and all elements are present. These data also demonstrated that MON 87708 does not contain detectable backbone sequences from the plasmid vector or T-DNA II sequences. The complete DNA sequence of the insert and adjacent genomic DNA sequence in MON 87708 confirmed the integrity of the inserted *dmo* expression cassette within the inserted sequences and identified the 5′ and 3′ insert-to-genomic DNA junctions. Furthermore, Southern blot analysis demonstrated that the insert in MON 87708 has been maintained through at least five generations of breeding, thereby confirming the stability of the insert over multiple generations.

Data Confirms the Safety of Expression Products in MON 87708

MON 87708 contains a *dmo* expression cassette that produces a single MON 87708 DMO precursor protein that is post-translationally processed into two forms of the dicamba mono-oxygenase (DMO) protein; referred to as MON 87708 DMO protein and MON 87708 DMO+27 protein. The active form of these proteins, necessary to confer dicamba tolerance, is a trimer comprised of three DMO monomers. In MON 87708, the trimer can be comprised of MON 87708 DMO protein, MON 87708 DMO+27 protein, or a combination of both. Unless specified otherwise in this document, MON 87708 DMO will refer to both proteins and all forms of the trimer, collectively.

A multistep approach was used to characterize MON 87708 DMO. This detailed characterization and assessment confirmed that MON 87708 DMO is safe for human and animal consumption. The assessment involved: 1) characterization of the physicochemical and functional properties of MON 87708 DMO; 2) quantification of MON 87708 DMO expression in MON 87708 plant tissues; 3) examination of the similarity of MON 87708 DMO to known allergens, toxins, or other biologically active proteins known to have adverse effects on mammals; 4) evaluation of the digestibility of MON 87708 DMO in simulated gastrointestinal fluids; 5) documentation of the history of safe consumption of MON 87708 DMO or its structural and functional homology to proteins that lack adverse effects on human or animal health; 6) evaluation of the stability of MON 87708 DMO after heat treatment; 7) investigation of potential mammalian toxicity in an acute mouse gavage; and 8) assessment of the potential for allergenicity, toxicity, and adverse biological activity of putative polypeptides encoded by the insert and flanking sequences.

MON 87708 DMO was fully characterized and the enzymatic activity was found to be specific for dicamba when tested using structurally similar soybean endogenous substrates. MON 87708 DMO was expressed in all tissues of MON 87708 at varying levels. MON 87708 DMO has no relevant amino acid sequence similarities with known allergens, gliadins, glutenins, or toxins that may have adverse effects on mammals. MON 87708 DMO was rapidly digested in simulated gastric and intestinal fluids. MON 87708 DMO was completely deactivated after heating at temperatures above 55°C. MON 87708 DMO was not acutely toxic and did not cause any observable adverse effects when tested in a mouse acute oral toxicity analysis. An open reading frame bioinformatic analyses of the junction site between the soybean genomic DNA and the insert confirm no relevant similarities exist between any putative polypeptides and known toxins or allergens. In addition, results from an IgE binding study using sera from soybean allergic individuals demonstrate MON 87708 does not pose an increased endogenous soybean allergenic risk compared to commercially available conventional reference soybean varieties. The safety assessment supports the conclusion that exposure to MON 87708 DMO poses no meaningful risk to human and animal health.

Food and Feed Safety Assessments of MON 87708 Demonstrate Compositional Equivalence to Conventional Crop

Detailed compositional analyses were conducted to assess whether levels of key nutrients and anti-nutrients in MON 87708 were comparable to levels present in the near isogenic conventional soybean control A3525 and several commercial reference soybean varieties. Seed and forage were harvested from five individual sites in which MON 87708 (treated with dicamba herbicide), the conventional control, and a range of commercial reference varieties were grown concurrently in the same field trial. The commercial reference varieties were used to establish a range of natural variability for the key nutrients and anti-nutrients in commercial soybean varieties that have a history of safe consumption. Nutrients assessed in this analysis included proximates (ash, carbohydrates by calculation, moisture, protein, and fat), fiber, amino acids (18 components), fatty acids (FA, C8-C22), and vitamin E (α-tocopherol) in seed; and proximates (ash, carbohydrates by calculation, moisture, protein, and fat) and fiber in forage. The anti-nutrients assessed in seed included lectin, phytic acid, raffinose, stachyose, trypsin inhibitors, and isoflavones (daidzein, genistein, and glycitein).

A combined-site analysis was conducted to determine statistically significant differences (5% level of significance) between MON 87708 and the conventional control. The results from the combined-site data were reviewed using considerations relevant to food and feed safety and nutritional quality. These considerations included assessments of: 1) the relative magnitudes of the difference in the mean values of nutrient and anti-nutrient components of MON 87708 and the conventional control, 2) whether the MON 87708 component mean value was 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 field trial, 3) analysis of the reproducibility of the statistically significant combined-site component differences at individual sites, and 4) assessing the differences within the context of natural variability of commercial soybean composition published in the scientific literature and in the International Life Sciences Institute (ILSI) Crop Composition Database.

Based on assessments of the levels of the components analyzed in MON 87708, it was determined that MON 87708 is compositionally equivalent to the conventional control and within the range of variability of the commercial reference varieties that were grown concurrently in the same field trial. There were either no statistically significant differences between MON 87708 and the conventional control or the differences observed in the

combined-site analysis were deemed to not be meaningful to food and feed safety or the nutritional quality of MON 87708. These results support the overall food and feed safety of MON 87708.

Conclusion

The data and information presented in this safety assessment demonstrate that the food and feed derived from MON 87708 are as safe and nutritious as conventional soybean, a familiar crop with a long history of safe consumption. The food and feed safety of MON 87708 was confirmed through multiple, well established lines of evidence:

1. The safety of the donor organism, *S. maltophilia*, based on its ubiquitous presence in the environment, presence in healthy individuals, and the incidental presence in foods without any adverse safety reports.

2. A detailed molecular characterization of the inserted DNA demonstrated a single, intact copy of the T-DNA stably inserted in a single locus within the soybean genome.

3. A history of safe use has been established for MON 87708 DMO. Data confirmed that MON 87708 DMO is unlikely to be a toxin or allergen based on extensive information collected. MON 87708 DMO was readily digestible in simulated gastric and simulated intestinal fluids, inactivated when exposed to heat, and showed no oral toxicity or cause any adverse effect in mice.

4. A compositional assessment of seed and forage confirmed that MON 87708 is compositionally equivalent to conventional soybean.

All data strongly support the conclusion that food and feed derived from MON 87708 will be as safe and nutritious as food and feed derived from conventional soybean.