# EXECUTIVE SUMMARY
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# Application to Food Standards Australia New Zealand for the Inclusion of Cotton MON 88701 in Standard 1.5.2 - Food Derived from Gene Technology

Submitted by:

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# EXECUTIVE SUMMARY

## MON 88701 Product Description

Monsanto Company has developed dicamba and glufosinate-tolerant cotton, MON 88701, that will permit in-crop applications of dicamba herbicide for the control of broadleaf weeds from pre-emergence to seven days pre-harvest and glufosinate herbicide for broad spectrum weed control from emergence through early bloom growth stage. Both herbicides provide a unique mode-of-action for effective weed management, including the control of glyphosate-resistant weeds. MON 88701 will be combined, through traditional breeding methods, with other approved herbicide-tolerant (*i.e.* glyphosate) events. MON 88701 may also be combined, through traditional breeding methods, with previously approved insect-protected cotton events. The in-crop use of dicamba and glufosinate herbicides, when used in combination with glyphosate herbicide, provides new weed management options in cotton, to control a broad spectrum of grass and broadleaf weed species and effective control of weeds resistant to several herbicide families.

MON 88701 contains a demethylase gene from *Stenotrophomonas maltophilia* that expresses a dicamba mono-oxygenase (DMO) protein to confer tolerance to dicamba herbicide and a bialaphos[1] resistance (*bar*) gene from *Streptomyces hygroscopicus* that expresses the phosphinothricin N-acetyltransferase (PAT) protein to confer tolerance to glufosinate herbicide. DMO protein rapidly demethylates dicamba to the herbicidally inactive metabolite 3,6-dichlorosalicylic acid (DCSA). DCSA has been previously identified as a metabolite of dicamba in cotton, soybean, livestock and soil. PAT (*bar*) protein acetylates the free amino group of glufosinate to produce the herbicidally inactive metabolite 2-acetamido-4-methylphosphinico-butanoic acid (N-acetyl glufosinate).

## History of Use of the Host and Donor Organisms

The *dmo* gene is derived from the bacterium *Stenotrophomonas maltophilia* strain DI-6, isolated from soil at a dicamba manufacturing plant. *S. maltophilia* is an aerobic, environmentally ubiquitous gram negative bacterium commonly present in aquatic environments, soil, and plants.

The *bar* gene is derived from the bacterium *Streptomyces hygroscopicus*, a saprophytic, soil-borne bacterium with no known safety issues. *Streptomyces* species are widespread in the environment and present no known allergenic or toxicity issues.

The ubiquitous presence of *S. maltophilia* and *S. hygroscopicus* in the environment, the presence of healthy individuals without causing infections, the incidental presence in foods without any adverse safety reports, and the lack of reported allergenicity establishes the safety of the donor organism.

[1] Bialaphos is a bacterial tripeptide composed of L-phosphinothricin (PPR) plus two alanines. *In vivo* the alanines are removed to produce L-PPT, a naturally occurring glutamate analogue with herbicidal activity through the inhibition of glutamine synthetase. Glufosinate is a synthetically produced racemic mixture of D and L-PPT.

**Nature of the Genetic Modification**

MON 88701 was developed through *Agrobacterium*-mediated transformation of hypocotyls from cotton variety Coker 130 utilising vector PV-GHHT6997. PV-GHHT6997 contains one T-DNA that is delineated by Left and Right Border regions. The T-DNA contains the *dmo* and *bar* expression cassettes. The *dmo* expression cassette is regulated by the *PC1SV* promoter, the *TEV* 5' leader sequence, and the *E6* 3' untranslated region. The chloroplast transit peptide CTP2 directs transport of the MON 88701 DMO protein to the chloroplast and is derived from *CTP2* target sequence of the *Arabidopsis thaliana shkG* gene. The *bar* expression cassette is regulated by the *e35S* promoter, the *Hsp70* leader, and the *nos* 3' untranslated region. After transformation, self pollination and segregation were used to select those plants containing a single homozygous copy of the T-DNA, including both the *dmo* and *bar* expression cassettes, resulting in the selection of MON 88701.

Molecular characterisation determined that MON 88701 contains one copy of the T-DNA at a single integration locus and all genetic elements are present. These data also demonstrated that MON 88701 does not contain detectable backbone sequences from the plasmid vector. The complete DNA sequence of the insert and adjacent genomic DNA sequences in MON 88701 confirmed the integrity of the inserted *dmo* and *bar* expression cassettes and identified the 5' and 3' insert to flank DNA junctions. Molecular characterisation analysis also demonstrated that the insert in MON 88701 has been maintained over five generations of breeding, thereby confirming the stability of the insert. Furthermore, results from segregation analyses showed inheritance and stability of the insert were as expected across multiple generations, which corroborates the molecular insert stability analysis determination that the MON 88701 T-DNA resides at a single chromosomal locus within the cotton genome.

**Characterisation of Novel Proteins or Other Novel Substances**

MON 88701 contains a *dmo* expression cassette that produces a dicamba mono-oxygenase protein referred to as MON 88701 DMO and a *bar* expression cassette that produces a phosphinothricin N-acetylase transferase protein (PAT) referred to as PAT (*bar*).

A multistep approach, in accordance with guidelines established by the Codex Alimentarius Commission, OECD, and the principles and guidance of the FDA's 1992 policy on foods from new plant varieties, was used to characterise the MON 88701 DMO and PAT (*bar*) proteins present in MON 88701. These steps include: 1) documentation of the history of safe use of the MON 88701 DMO and PAT (*bar*) proteins and their structural and functional homology with proteins that lack adverse effects on human or animal health; 2) characterisation of the physicochemical and functional properties of MON 88701 DMO and PAT (*bar*) proteins; 3) quantification of MON 88701 DMO and PAT (*bar*) expression in plant tissues; 4) examination of the similarity of MON 88701 DMO and PAT (*bar*) proteins to known allergens; 5) evaluation of the digestibility of MON 88701 DMO and PAT (*bar*) in simulated gastrointestinal fluids; 6) evaluation of the stability of the MON 88701 DMO and PAT (*bar*) proteins in response to typical food/feed preparation conditions such as heat treatment; 7) examination of the similarity

of MON 88701 DMO and PAT (*bar*) to known toxins or other biologically active proteins known to have adverse effects on mammals; 8) investigation of potential mammalian toxicity through an animal assay and calculating margins of exposure; and 9) examination of the similarity of putative polypeptides encoded by the insert and flanking sequences to known allergens and toxins or other biologically active proteins known to have adverse effects on mammals. The safety assessment supports the conclusion that dietary exposure to MON 88701 DMO and PAT (*bar*) proteins derived from MON 88701 poses no meaningful risk to human or animal health.

**Potential Toxicity and Allergenicity of Novel Proteins**

A history of safe use has been demonstrated for both MON 88701 DMO and PAT (*bar*) proteins. MON 88701 DMO was fully characterised and the enzymatic activity was found to be specific for dicamba when tested using structurally similar cotton endogenous substrates. The specificity of PAT proteins has been extensively documented in the literature. Netiehr protein has relevant amino acid sequence similarities to known allergens, gliadins, glutenins, or toxins that may have adverse effects on mammals. MON 88701 DMO and PAT (*bar*) were each rapidly digested in simulated gastric and intestinal fluids. Both proteins lost significant functional activity at temperatures well below those used in cottonseed processing to generate cottonseed meal, oil, and linters. MON 88701 DMO was completely deactivated after heating at temperatures above 55 °C and PAT (*bar*) lost greater than 90% functional activity at temperatures of 75 °C and above. Neither MON 88701 DMO nor PAT (*bar*) were acutely toxic and did not cause any observable adverse effects when tested in mouse acute oral toxicity analyses. In addition, the only fractions derived from cottonseed that are used in food applications are oil and linters, which contain undetectable and negligible amounts of protein, respectively. Therefore, MON 88701 DMO and PAT (*bar*) proteins comprise a very low, non-detectable portion of the total protein present in food derived from MON 88701. Based on a history of safe use, an apparent absence of hazard, and lack of dietary exposure a dietary risk assessment for these proteins is considered unnecessary. An open reading frame bioinformatic analysis of the junction site between the cotton genomic DNA and the insert confirms no relevant similarities exist between any putative polypeptides and known toxins or allergens. The safety assessment supports the conclusion that exposure to MON 88701 DMO and PAT (*bar*) from MON 88701 poses no meaningful risk to human and animal health.

**Toxicity of Novel Herbicide Metabolites in GM Herbicide-Tolerant Plants**

DMO protein rapidly demethylates dicamba to the herbicidally inactive metabolite 3,6-dichlorosalicylic acid (DCSA). DCSA has previously been identified as a metabolite of dicamba in cotton, soybean, livestock and soil and is not persistent in the environment, has low potential for leaching to ground water and has not been found to be toxic to organisms in the environment.

PAT (*bar*) protein acetylates the free amino group of glufosinate to produce non-herbicidal N-acetyl glufosinate, a well known metabolite in glufosinate tolerant plants. Glufosinate and N-acetyl glufosinate are not known to be toxic.

## Compositional Analyses of the GM Food

Detailed compositional analysis, in accordance with OECD guidelines, were conducted to assess whether levels of key nutrients and ant-nutrients in MON 88701 cottonseed were comparable to levels in the conventional cotton Coker 130, with similar background genetics, and several commercial reference cotton varieties.

Cottonseed were harvested from eight sites in which MON 88701 (treated sequentially with glufosinate and dicamba herbicides), 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 of the key nutrients and anti-nutrients in commercial cotton varieties that have a long history of safe consumption. Nutrients assessed in this analysis included proximate (ash, fat, moisture, protein, and carbohydrates by calculation), calories by calculation, acid detergent fibre (ADF), neutral detergent fibre (NDF), crude fibre (CF), total dietary fibre (TDF), amino acids (AA, 18 components), fatty acids (FA, C8-C22), minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc) and vitamin E. The key anti-nutrients assessed included gossypol and cyclopropenoid fatty acids (CPFA).

Combined-site analyses were conducted to determine statistically significant differences ($p<0.05$) between herbicide-treated MON 88701 and the conventional control cottonseed samples. Any significant differences noted from the combined-site statistical comparison were assessed using considerations relevant to the safety and nutritional quality of MON 88701 when compared to the conventional control. Considerations used to assess the relevance of each combined-site statistically significant difference included: 1) the relative magnitude of the difference in the mean values of nutrient and anti-nutrient components between MON 88701 and the conventional control; 2) whether the MON 88701 component mean value is 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 trial; 3) evaluation of the reproducibility of the statistical ($p<0.05$) combined-site component differences at individual sites; and 4) an assessment of the differences within the context of natural variability of commercial cotton composition published in the scientific literature and in the International Life Sciences Institute (ILSI) Crop Composition Database.

Based on the analysed nutrient and anti-nutrient levels, herbicide-treated MON 88701 is compositionally equivalent to conventional cotton and therefore the food and feed safety and nutritional quality of this product is comparable to that of the conventional cotton. These results support the overall food and feed safety of MON 88701.

## Conclusion

All data and information contained within this document strongly support the conclusion that food and feed derived from MON 88701 and its progeny will be as safe and nutritious as food and feed derived from conventional cotton.