

Determination of the Safety of Monsanto's  
**Herbicide (Dicamba) Tolerant Soybean MON 87708**  
For Direct Use as Food and Feed and for Processing

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**Food and Feed Safety**

The product dossier of Herbicide (Dicamba) Tolerant Soybean MON 87708 was reviewed for safety and nutritional differences compared with the conventional soybean. The review was focused on any new or altered expression trait and changes in composition and nutritional content or value relative to the conventional soybean. After thorough evaluation on the safety assessment, the following conclusions were made: Herbicide (Dicamba) Tolerant MON 87708 soybean is as safe as its conventional counterpart taking into account the safety and nutritional quality of MON 87708. Herbicide (Dicamba) Tolerant MON 87708 soybean is safe to humans, animals and as nutritious as conventional soybean.

A biosafety permit for Herbicide (Dicamba) Tolerant MON 87708 and all progenies derived from crosses of the product with any conventional soybean containing approved-biotech events for direct use was issued to Monsanto Philippines Inc., on May 5, 2014. The validity of the permit is five years.

*This approval is for direct use as food and feed and for processing of Herbicide (Dicamba) Tolerant MON 87708 soybean in the Philippines. Food and feed use of Herbicide (Dicamba) Tolerant MON 87708 soybean and its by-products is therefore authorized as of May 5, 2014. The biosafety permit (No. 14-077) for direct use stated that "Herbicide (Dicamba) Tolerant MON 87708 soybean and all progenies from crosses of this product except when stacked with other biotech traits has undergone satisfactory assessment and found to be as safe as conventional soybean and can be a substitute for its traditional counterpart as food and feed and for processing"*

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**I. Brief Identification of the Genetically Modified Organism (Living Modified Organism)**

Designation:	MON 87708 soybean
Applicant:	Monsanto Philippines 23 <sup>rd</sup> Floor Tower II Insular Life Corporate Centre Insular Life Drive Filinvest Corporate City Alabang, Muntinlupa City Philippines 1781
Plant Species:	
Name:	Soybean ( <i>Glycine max</i> (L) Merr.)
Parent Material:	Soybean A3525
Center of Origin:	Southeast Asia; wild soybean species endemic in China, Korea, Japan and Taiwan

Toxic Factors/Allergen(s): Trypsin inhibitors, lectins, Isoflavone (daidzein, genistein, glycitein) stachyose, raffinose and phytic acid.

Trait Description: Herbicide (Dicamba) Tolerant soybean

Trait Introduction Method: *Agrobacterium tumefaciens* mediated transformation

Donor Organism: *Stenotrophomonas maltophilia* (*S. maltophilia*) expressing a mono-oxygenase (*dmo*) that rapidly demethylates 3,6-dichloro-2-methoxybenzoic acid (dicamba) rendering it inactive metabolite, 3,6-dichlorosalicylic acid (DCSA)

Pathogenicity: The donor organism, *S. maltophilia*- an aerobic bacterium found in the environment and is found in the variety of foods and feeds, is safe. It can be found in healthy individuals without causing any harm to human health, and infections in humans caused by *S. maltophilia* are extremely uncommon. *S. maltophilia* has not been reported to be a source of allergens

Proposed Use: For direct use as food and feed and for processing

## **II. Background Information**

Monsanto Philippines has developed biotechnology-derived soybean MON 87708 that is tolerant to dicamba herbicide by producing MON 87708 DMO (active form of DMO in MON 87708, a trimer comprised of three monomers). The DMO trimer can be comprised of MON 87708 DMO protein, MON 87708 DMO+27 protein, or a combination of both proteins. On 18 May 2011, Monsanto submitted an application to the Bureau of Plant Industry (BPI) requesting for biosafety permit under Department of Agriculture Administrative Order No. 8, series of 2002 for Dicamba Tolerant Soybean MON 87708.

Monsanto has provided data or information on a detailed description of the transformation method, the source of the done gene, molecular characterization of MON 87708 soybean and levels of expression in the plant. The novel protein. MON 87708 DMO was identified, characterized and compared to the original bacterial proteins, including an evaluation of their potential toxicity to livestock and non-target organisms. Relevant scientific publications were supplied.

Soybean MON 87708 has been evaluated according to BPI's safety assessment by concerned agencies: Bureau of Animal Industry (BAI), BPI, Bureau of Agriculture and Fisheries Standards (BAFS) and a Scientific and Technical Review Panel (STRP) members. The process involved an intensive analysis of the nature of the genetic modification with a consideration of general safety issues, toxicological and nutritional issues associated with the modified soybeans.

The petitioner/applicant published the said application in two (2) widely circulated newspapers namely: Malaya Business Insight and The Daily Tribune on 13 January 2014 for public comment/review. During the 30-day comment period, BPI had not received comment on the said application.

Review of results of evaluation by the BPI Biotech Core Team completed the approval process.

### **III. Description of Novel Protein (Introduced Traits)**

MON 87708 was developed through *Agrobacterium tumefaciens*-mediated transformation of conventional soybean A3525 meristem tissue with 2T-DNA plasmid vector PV-GMHT4355, which contains two separate T-DNAs (T-DNA I and T-DNA II) that are each delineated by Left and Right Border regions. Transfer DNA (T--DNA refers to DNA that is transferred to the plant during transformation).

The first T-DNA, designated as T-DNA I, contains the *dmo* coding sequence for the DMO from the bacterium *Stenotrophomonas maltophilia* strain DI-6 isolated from soil. The presence of MON87708 DMO confers tolerance to dicamba. The second T-DNA, designated as T-DNA II, contains the *cp4 epsps* coding sequence from *Agrobacterium* sp. strain CP4 encoding CP4 EPSPS conferring tolerance to glyphosate and was used as a selectable marker during the transformation selection process. Subsequently, due to self-pollination and segregation, *cp4 epsps* coding sequence was eliminated.

#### **Safety of the Expressed Proteins**

A history of safe use has been established for MON 87708 DMO. MON 87708 DMO lacks structural similarity to known allergens or toxins known to have adverse effects on mammals based on bioinformatics analyses. According to guidelines adopted by the Codex Alimentarius Commission for the assessment of the potential allergenicity of introduced proteins, the allergenic potential of an introduced protein is assessed by comparing the biochemical characteristics of the introduced protein to characteristics of known allergens. The assessment of potential toxicity of an introduced protein is based on comparing the biochemical characteristics of known toxins.

MON 87708 DMO was detected in all plant tissues assayed at a number of times points during the growing season. Harvested seed is the most relevant tissue analyzed for an allergenicity assessment because it can be consumed directly. MON 87708 DMO is present at very low levels (0.011%) in MON 87708 seed and constitutes a very small portion of the total protein present in food and feed derived from MON 87708. This low percent of MON 87708 DMO in relation to the total protein reduces the potential for the protein to be an allergen.

The digestive fate of DMO was assessed during assays with both simulated gastric fluid (SGF) containing pepsin and simulated intestinal fluid (SIF) containing pancreatin. Digestibility of MON 87708 in SGF was assessed using the SDS-PAGE and immunoblot methods. It was also evaluated by Western blot. The digestion of MON 87708 DMO in SIF was separated by SDS-PAGE. A Western blot to determine the limit of detection (LOD) of MON 87708 DMO was performed concurrently with the SIF assay.

Visual examination of the colloidal Brilliant Blue G stained Tris-glycine 8% gel showed that MON 87708 DMO was digested to less than 2% of total protein loaded in SGF within 30 seconds. No fragments corresponding to MON 87708 DMO were

observed in the 30 second digestion sample. A diffuse faint staining band of ~21 kDa protein fragment that was observed for all time points was sequenced and found not to match any of the MON 87708 DMO sequences.

Visual examination of the western blot confirmed that MON 87708 DMO was digested to less than 1.5% of the total protein loaded in SGF within 30 seconds. No fragments corresponding to MON 87708 DMO were observed.

The methods used and the data generated were sufficient to show that DMO was digested in SGF and SIF. The rapid digestion of MON 87708 DMO SGF and SIF indicates that it is highly unlikely that MON 87708 DMO will pose any safety concern to human and animal health.

The effect of heat treatment on the activity of MON 87708 DMO was evaluated using a functional activity assay and SDS-PAGE. The functional activity of MON 87708 was unaffected at 25<sup>0</sup>C and 37<sup>0</sup>C for 15 and 30 minutes. After incubation at 55<sup>0</sup>C or higher for 15 minutes or more, the functional activity was below the limit of quantification (LOQ) of the assay functional activity of MON 87708 DMO had been lost during heating. Since soybean is processed using heat treatment usually higher than 55<sup>0</sup>C, it is expected that this will lead to a loss in active MON 87708 DMO in products such as soybean meal.

An acute oral mouse toxicity study was done on MON 8778 DMO and considered sufficient to evaluate the toxicity of MON 87708 DMO, since its sequence is not similar to any anti-nutritional proteins or any known protein toxins. There were no treatment related effects on survival, clinical observations, body weight, weight gain, food consumption or gross pathology. No Observable Adverse Effect level (NOAEL) for MON 87708 DMO was placed at 140mg/kg bw, the highest dose tested.

In conclusion, 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 effects on mice.

#### **IV. Nutritional Composition (Compositional Analysis)**

The compositional equivalence of MON 87708 seed and forage to conventional soybean was analyzed through a comprehensive comparative assessment of the levels of key nutrients and anti-nutrients in seed and forage. Nutrients assessed included proximate (ash, carbohydrates, moisture, protein and fat), fiber, amino acids (18 components), fatty acids and vitamin E-alpha tocopherol in seed and proximate and fiber in forage.

Result of comprehensive assessment is demonstrated that the levels of key nutrients in seed and of the key nutrients in forage of MON 87708 were within the range of natural variability of conventional commercial reference soybean varieties and MON 87708 is compositionally equivalent to conventional soybeans. The results further showed no health risk concern with the consumption of MON 87708 soybean.

## **V. Anti-Nutritional Factors**

MON 87708 was compared to conventional soybean in eight anti-nutrient components. Statistically significant differences were observed in four namely, phytic acid, raffinose, stachyose, and daidzein. The levels of these antinutrients in seed of MON 87708 were within the range of natural variability of conventional commercial reference soybean varieties. The results further showed no health risk concern with the consumption of MON 87708 soybean.

Soybean meal produced from MON 87708 compared to meal produced from conventional soybean through a broiler study showed no biologically meaningful differences in broiler performance, carcass yield or meat composition between the two groups. There was a comparable nutritional wholesomeness of the two diets.

## **VI. Regulatory Decision**

After viewing the scientific data and information relevant to the application of Monsanto Philippines, Inc, it is concluded that MON 87708 soybean and all progenies from crosses of this product except when stacked with other biotech traits has undergone satisfactory biosafety assessment and found to be as safe as conventional soybean and can be a substitute for its traditional counterpart and is therefore approved for direct use as food and feed and for processing.

Dicamba-tolerant MON 87708 soybean shall be imported solely for direct use as food and feed and for processing and not for field testing or propagation. Monsanto Philippines, Inc shall take appropriate measures to protect human and animal health and the environment and prevent a recurrence should accidental, unintentional reproduction occurs. A plant quarantine officer and his duly authorized representatives shall be allowed access during regular business hours to the facility where the regulated article is located and to any records relating to the importation of the regulated article. Monsanto shall duly inform the public of this approval by way of publishing in any one (1) of the top three (3) leading newspapers in the country. A copy of such publication shall be submitted to the BPI within ten (10) days after publication. This product is covered by conditions for approval as provided in Department of Agriculture Memorandum Circular No. 8, Series of 2003.