

**Determination of the Safety of Monsanto Philippines’
MON 87701 Soybean (Insect Protected)
For Direct Use as Food and Feed or for Processing**

Food and Feed Safety

The product dossier MON 87701 Soybean 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: MON 87701 soybean is as safe as its conventional counterpart taking into its dietary source and nutritional impact of any changes in nutritional value. MON 87701 soybean is safe to humans, animals and as nutritious as conventional soybean. Expression of the *Bacillus thuringiensis* (*Bt*) subsp. *kurstaki* insecticidal protein Cry1Ac provides protection from feeding damage by target lepidopteran pests, including velvetbean caterpillar (*Anticarsia gemmatalis*), soybean looper (*Pseudoplusia includens*), soybean axil borer (*Epinotia aporema*), and sunflower looper (*Rachiplusia nu*). Reduction in the use of insecticides used to control the lepidopteran pests and beneficial from the reduction of damage caused by these insects are expected for the growers who will be using MON 87701. Thus, reduction of insecticide application is also expected to benefit the environment.

A biosafety permit for Soybean MON 87701 (No: 12-057) and all progenies from crosses of the product except when stacked with other biotech traits was issued to Monsanto Philippines, Inc. on May 16, 2012. The permit was valid for five years, subject to the terms and conditions set forth in DA Administrative Order No. 8, Series of 2002 as amended by DA Administrative Order No. 22, Series of 2007. The said MON 87701 was included in the Approval Registry prepared by the Department of Agriculture- Bureau of Plant Industry.

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

Designation:	MON 87701, MON-877Ø1-2
Applicant:	Monsanto Philippines, Inc. 7 th Floor Ayala Life-FGU Center Alabang-Zapote Road cor., Acacia Ave. Madrigal Business Park, Alabang, Muntinlupa City
Plant Species:	
Soybean:	Soybean (<i>Glycine max</i> (L.) Merr.)
Parent Material :	Soybean variety A5547
Center of Origin:	Southeast Asia; wild soybean species endemic in China, Korea, Japan, Taiwan
Toxic Factors/Allergen(s):	Trypsin inhibitors, lectins, isoflavones (daidzein, genistein and glycitein), stachyose, raffinose, and phytic acid
Trait Description:	Resistance to lepidopteran pests of soybean including velvetbean caterpillar (<i>Anticarsia gemmatalis</i>), soybean

looper (*Pseudoplusia includens*), soybean axil borer (*Epinotia aporema*), and sunflower looper (*Rachiplusia nu*).

Trait Introduction Method: *Agrobacterium tumefaciens*-mediated transformation

Donor Organism: *Bacillus thuringiensis* subsp. *kurstaki*, source of insecticidal protein Cry1Ac which is a gram-positive bacterium that is commonly found in soil and has been used commercially in agriculture for pest control

Pathogenicity: *Bacillus thuringiensis* subsp. *kurstaki*, the donor organism of Cry1Ac protein, is not a known human or animal pathogen and is not known to cause allergic reactions in humans. There is no evidence that *Bt* is pathogenic to humans and it is not known to produce toxins or allergens.

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

II Background Information

Monsanto Philippines, Inc. has developed biotechnology-derived, insect-protected soybean MON 87701 that produces the Cry1Ac insecticidal crystal (δ -endotoxin) protein derived from *Bacillus thuringiensis* (*Bt*) subsp. *kurstaki*. Cry1Ac provides protection from feeding damage caused by targeted lepidopteran pests, including the velvetbean caterpillar (*Anticarsia gemmatilis*), soybean looper (*Pseudoplusia includens*), soybean axil borer (*Epinotia apomera*), and sunflower looper (*Rachiplusia nu*) (Adang et al., 1985), and will reduce or replace current insecticide applications in tropical and subtropical production regions, where these insects cause significant plant damage and yield loss.

On October 8, 2009, Monsanto Philippines, Inc. submitted an application to the Bureau of Plant Industry requesting for biosafety permit under A.O. #8 for soybean which has been genetically modified for insect protection.

Monsanto Philippines, Inc. has provided information on the safe history of use of the crop, the source of the donor gene, the molecular characterization of MON 87701 soybean, the stability of the inserted genetic elements, characterization and expression levels of Cry1Ac proteins produced in the MON 87701 soybean plant, safety of the protein including lack of any allergenicity or toxicity characteristics associated with Cry1Ac and MON 87701 as well as the nutrient composition of the soybean grain, forage and grain processed fractions, and overall food and feed safety of MON 87701 soybean plants. Relevant scientific publications were also supplied.

MON 87701 Soybean has been evaluated according to BPI's safety assessment by concerned agencies: Bureau of Animal Industry (BAI), Bureau of Plant Industry (BPI), Bureau of Agriculture Fisheries and Product Standards (BAFPS) and a Scientific Technical Review Panel (STRP). The process involved an intensive analysis of the nature of the genetic modification with a consideration of general safety issues, toxicological, nutritional and environmental issues associated with the modified soybeans.

The petitioner/applicant published the said application in two (2) widely circulated newspapers (Malaya Business and The Daily Tribune) on February 9, 2012 for public comment/review. BPI did not received any comments during the 30-day comment period in regards to the of MON 87701 Soybean application for direct use as food and feed or for processing.

Review of results of evaluation by the BPI Biotech Core Team in consultation with DA-Biotechnology Advisory Team (DA-BAT) completed the approval process.

III Description of Novel Protein (Introduced Traits)

The Cry1Ac insecticidal crystal (δ - endotoxin) protein that has been produced by soybean MON 87701 is derived from *Bacillus thuringiensis* (Bt) subsp. *kurstaki*. The *cry1Ac* gene was transferred into the genome of soybeans cells using *Agrobacterium tumefaciens*-mediated transformation with PV-GMIR9, which is a binary vector containing two transfer DNAs (2T-DNAs) the first T-DNA, designated as T-DNA I, contains the *cry1Ac* gene cassette. The second T-DNA, designated as T-DNA II contains the *cp4 epsps* gene cassette. Both T-DNAs were inserted into the soybean genome during the transformation. The *cp4 epsps* gene was used as a selectable marker to select transformed cells and plants. After the transformed cell and subsequent plants were identified, the selectable marker gene was no longer used. A traditional breeding process was deployed to isolate plants that contain only the *cry1Ac* expression cassette (T-DNA I), thereby, producing marker-free MON 87701 plants.

Safety of the Expressed Proteins

The molecular characterization data established that the genetically modified soybean MON 87701 contains one copy of an intact *cry1Ac* expression cassette. No other parts of the plasmid used for transformation are present in the transformed plant. Results of the bioinformatic analysis of the 5' and 3' flanking sequences and ORFs spanning the newly created DNA junctions did not indicate any safety concern. The stability of the inserted DNA was confirmed over several generations, the insertion did not result in unintended gene products with similarity to known allergens or toxins and a Mendelian inheritance pattern was demonstrated.

A detailed characterization and safety evaluation of the newly expressed Cry1Ac protein confirm that it is safe for human and animal consumption. Bioinformatics assessments showed that Cry1Ac does not share amino acid sequence similarities with known allergens, gliadins, glutenins, or protein toxins that have adverse effects on mammals. Digestive fate experiments demonstrated that the full-length Cry1Ac is rapidly digested in stimulated gastric fluid (SGF), although a small, transiently stable fragment is formed, and that it is highly unlikely that the Cry1Ac and its fragment will reach absorptive cells of the intestinal mucosa, resulting in low to no allergic risk. A heat stability experiment demonstrated that the immunodetectable level of the Cry1Ac protein in harvested seed of MON 87701 was significantly impacted by heat treatment. These results demonstrate that the heating of ground soybean seed, in a manner that stimulates the use of soybean flour in food manufacturing, results in the loss of immunodetectable Cry1Ac protein.

The safety of Cry1Ac is further confirmed by history of safe use, low toxicity potential, and no anticipated risk to humans and animals from its presence in the diet. Cry1Ac is a member of the family of Bt Cry proteins that have been used in agriculture as microbial pesticides for over 50 years with no evidence of adverse effects to human or animal health. Since 1996, a number of insect-resistant biotechnology crops expressing Bt Cry proteins have been commercialized. The data indicate that food and feed derived from MON 87701 containing Cry1Ac protein are as safe for consumption as the food and feed derived from conventional soybean.

IV Nutritional Composition (Compositional Analysis)

Studies demonstrated that the mean and range values for proximate analyses for moisture, ash, protein, total fat, dietary fiber, carbohydrates, amino acids, fatty acids; vitamins (tocopherols) showed comparable ranges with commercial soybean.

The nutritional equivalence of MON 87701 soybean to conventional soybean was confirmed in feeding studies with broiler chickens. The studies showed that MON 87701 and the commercial variety are substantially equivalent in comparison.

Thus MON 87701 can be used in food and feed formulations and 100% substitute for commercial soybean.

V Anti-Nutritional Factors

Although soybean does not produce any known toxic compounds, it does contain antinutrients with very low amount and are below the thresholds considered to raise a food safety concern. These include trypsin inhibitors, lectins, isoflavones (daidzein, genistein and glycitein), stachyose, raffinose and phytic acid. However, the amount of anti-nutrients present in MON 87701 soybean fell within the range found in conventional standard varieties.

VI Regulatory Decision

After viewing the scientific data and information relevant to the application of Monsanto Philippines, Inc. it is concluded that MON 87701 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 substitute for its traditional counterpart for direct use as food and feed or for processing.

The regulated article shall be imported solely for direct use as food and feed or 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. Monsanto shall duly inform the public of this approval by the way of publishing in any one (1) of the top three (3) leading newspapers in the country that imports of this product is covered by conditions for approval as provided in Department of Agriculture Memorandum Circular No. 8, Series of 2003.