

**Determination of the Safety of Monsanto and Dow AgroSciences’
Combined Trait Product Corn: MON89034 x 1507 x MON88017 x 59122-7
for Direct use as Food, Feed, or Processing**

Food and Feed Safety

The product dossier on Monsanto and Dow AgroSciences’ combined trait corn: MON89034 x TC1507 x MON88017 x DAS59122-7 *was* reviewed for safety based on three major issues/concerns regarding stacked genes from different sources namely a) gene interaction; b) effect on metabolic pathways and c) differential gene expression due to stacking by conventional breeding.

A biosafety notification for combined trait product corn: MON89034 x TC1507 x MON88017 x DAS59122-7 and all progenies derived from crosses of this product for direct use as food, feed or for processing was issued to Monsanto Philippines Inc. and Dow AgroSciences on February 9, 2010. The notification is valid for five years and shall expire on February 8, 2015 subject to the terms and conditions set forth in DA Administrative Order No. 8, Series of 2002, and No.22, series of 2007, Memorandum Circular No. 6 and 8, Series of 2004 and Series of 2007. The said combined trait product will be included in the List of Approval Registry being prepared by the Department of Agriculture – Bureau of Plant Industry.

This approval is for use as Food, Feed or for Processing only. This does not include cultivation of combined trait product corn: MON89034 x TC1507 x MON88017 x DAS59122-7 in the Philippines. Food and Feed use of combined trait product corn: MON89034 x TC1507 x MON88017 x DAS59122-7 and its by-products is therefore authorized as of February 9, 2010. The biosafety notification (No. 10-028) stated that combined trait product corn: MON89034 x TC1507 x MON88017 x DAS59122-7 is as safe for human food, livestock feed or for processing as its conventional counterparts.

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

Designation: Combined trait product corn: MON89034 x TC1507 x MON88017 x 59122-7

Applicant: MONSANTO PHILIPPINES, INC.
23rd Floor Tower II,
Insular Life Corporate Centre,
Insular Drive, Filinvest Corporate City
Alabang Muntinlupa City Philippines 1781

DOW AGROSCIENCES B.V PHILIPPINES
23rd Floor 6750 Ayala Avenue, Makati City Philippines

Plant Species:

Name: Corn (Zea mays)

Parent Material:	Inbred corn lines (and/or isolines) developed and produced by Monsanto and Dow AgroSciences
Center of Origin:	Corn originated in the highlands of Mexico 7,000 to 10,000 years ago.
Toxic Factors/Allergen(s):	Corn is not a common allergenic food. Allergies to corn are rare (less than six in over one hundred patients referred to as pediatric allergy specialty center for grain allergy). Anti-nutrients including trypsin inhibitor, phytic acid, and secondary metabolites such as raffinose, ferulic acid and p-coumaric acid are present in low amount in corn. 2-4 dihydroxy-7-methoxy-2H-1, 4 benzoxazin-3(4H)- one (DIMBOA) is a potential toxicant but declines rapidly as the plant grows
Trait Description:	Insect Resistant and Herbicide Tolerant
Trait Introduction Method:	Conventional breeding among the four genetically modified corn events (MON89034, TC1507, MON88017 and DAS59122)
Donor Organisms:	<p><i>Bacillus thuringiensis</i> subsp. <i>Kurstaki</i> - a source of (Bt) <i>cry1A.105</i> and <i>cry2Ab2</i> genes which confer resistance to lepidopteran pest insects.</p> <p><i>Bacillus thuringiensis</i> subsp. <i>aizawai</i> - a source of <i>cry1F</i> gene which confers resistance to lepidopteran pest insects.</p> <p><i>Streptomyces viridochromogenes</i> -a source of <i>pat</i> gene which confers tolerance to herbicide (glufosinate).</p> <p><i>Bacillus thuringiensis</i> subsp. <i>Kumamotoensis</i> -a source of the <i>cry3Bb1</i> gene which confers protection against corn rootworm.</p> <p><i>Agrobacterium tumefaciens</i> strain CP4 -a source of the <i>cp4 epsps</i> gene which confers tolerance to herbicide (glyphosate).</p> <p><i>Bacillus thuringiensis</i> strain PS149B1- a source of <i>cry34Ab1</i>, <i>cry35Ab1</i> genes which confers protection against coleopteran pests insects.</p>
Pathogenicity:	The donor organism for the Cry1A.105 and Cry2Ab2 proteins, <i>Bacillus thuringiensis</i> has been used commercially in the US for over four decades to produce microbial pesticides. Cry proteins have a history of safe use since 1958 as active ingredients either in Bt microbial pesticides or in biotechnology derived food and feed crops. The Cry1A.105 protein produced in MON 89034 is structurally and functionally similar to Cry1A protein produced in a number of biotechnology-derived crops (e.g Yieldgard®)

corn borer corn, Bollgard® cotton and Bollgard II® cotton) that have a history of safe use.

Bacillus thuringiensis var. *aizawai* is found naturally in soil worldwide. No known mammalian health effects have been reported. It has been in commercial use as a microbial pesticide on food crops, including fresh vegetables, for over 30 years.

Streptomyces viridochromogenes is a non-pathogenic microorganism commonly found in the soil environment. There are no known adverse pathogenic or toxicological effects reported.

Bacillus thuringiensis subsp. *kumamotoensis* is a spore-forming, gram-positive bacterium found naturally in soil. Bt strains have been used to produce products with insecticidal activity. Many Bt strains have shown to produce protein crystals or inclusion bodies that are selectively toxic to certain species of insect pests.

Agrobacterium sp. strain CP4 is not known for human and animal pathogens and not commonly allergenic. There is no known population of individuals sensitized to bacterial proteins.

The Bt genes cry34Ab1 and cry35Ab1 originates from *Bacillus thuringiensis* strain PS149B1 which comes from a diverse group of gram-positive, spore-forming microorganisms which produces insecticidal proteins that are very selective in toxicity to specific organisms. Decades of safety testing on Bt proteins demonstrate the lack of toxicity to humans and animals, and the absence of adverse effects on non-target organisms and environment.

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

II. Background Information

Monsanto company and Dow AgroSciences have developed a combined trait product corn: MON89034 x TC1507 x MON88017 x DAS59122-7 with insect (Lepidoptera and Coleoptera) resistant and herbicide (Glyphosate and Glufosinate) tolerant.

On June 4, 2009, Monsanto Philippines Inc., and Dow AgroSciences B.V. Philippines have filed an application with attached technical dossiers to the Bureau of Plant Industry for a biosafety notification for direct use as food, feed or for processing under Administrative Order (AO) No. 8 Part 5 for stacked trait product corn: MON89034 x TC1507 x MON88017 x DAS59122-7 which was developed by conventionally crossing four genetically modified corn events (MON89034, TC1507 x MON88017 x DAS59122-7) for insect (Lepidoptera and Coleoptera) resistance and herbicide (Glyphosate and Glufosinate) tolerance.

A safety assessment of combined trait product corn: MON89034 x TC1507 x MON88017 x DAS59122-7 was conducted as per Department of Agriculture Administrative Order No. 8, Series of 2002 and No. 22, Series of 2007, Memorandum Circulars Nos. 6 and 8, Series of 2004 and No.6, Series of 2007. The focus of risk assessment is a) gene interaction b) effect on metabolic pathways from the transgenes and c) differential gene expression due to stacking by conventional breeding.

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

III. Description of Novel (Introduced) Traits

MON 89034 was developed through Agrobacterium-mediated transformation of corn to produce the Bt insecticidal proteins Cry1A.105 and Cry2Ab2 using the binary plasmid vector PV-ZMIR245. The Cry1A.105 and Cry2Ab2 proteins are insect control proteins and act through a toxic action in the midgut of specific lepidopteran insects, such as the *Ostrinia nubilalis* (European corn borer; ECB), *Ostrinia furnacalis* (Asian corn borer, ACB), *Helicoverpa zea* (corn earworm; CEW), *Spodoptera frugiperda* (fall armyworm; FAW), and *Spodoptera litura* (common cutworm; CW). The Cry1A.105 is a chimeric protein consisting of domains I and II from Cry1Ab or Cry1Ac, a substantial portion of domain III from Cry1F, and the C-terminal domain from Cry1Ac. The Cry1Ac and Cry1Ab proteins are derived from the common soil bacterium *Bacillus thuringiensis* (Bt) subsp. *kurstaki* and Cry1F is derived from Bt subsp. *aizawai*. The Cry2Ab2 protein is derived from Bt subsp. *kurstaki*. The combination of the two insecticidal proteins provides enhanced insect control relative to first generation products and offers an effective insect-resistance management tool.

TC1507 was produced via microprojectile bombardment of corn cells, using a PmeI-linearized portion of plasmid PHP8999 containing two expression cassettes: cry1F and pat. TC1507 produces the Bt var. *aizawai* Cry1F protein to selectively control European corn borer larvae and other lepidopteran insect pests. In addition, TC1507 produces the phosphinothricin acetyl transferase (PAT) protein from *Streptomyces viridochromogenes*, to confer tolerance to glufosinate-ammonium, the active ingredient in Liberty herbicide.

MON 88017 was developed by the Agrobacterium-mediated transformation using the plasmid vector PV-ZMIR39. MON 88017 contains a single, intact DNA insert with two expression cassettes, cry3Bb1 and cp4 epsps. The first expression cassette, cry3Bb1 produces Cry3Bb1 protein, an insect control protein which acts through a toxic action in the midgut of corn rootworm larvae, i.e., coleopteran species *Diabrotica virgifera virgifera*, *D. barberi*, and *D. virgifera zea*. The second expression cassette, cp4epsps produces CP4EPSPS protein which confers tolerance to glyphosate, the active ingredient in Roundup® family of agricultural herbicides. The cry3Bb1 gene was derived from the common soil bacterium *Bacillus thuringiensis* subsp. *kumamotoensis* and was introduced into corn, along with the cp4 epsps gene, which was derived from the Agrobacterium sp., strain CP4, a common soil-borne bacterium, to generate MON 88017.

Corn 59122 was produced by the Agrobacterium-mediated transformation of corn cells using the plasmid PHP17662, which contained three expression cassettes: cry34Ab1, cry35Ab1, and pat. Corn 59122 produces the Bt strain PS149B1 Cry34Ab1 and Cry35Ab1 binary proteins (Cry34/35Ab1) to protect against coleopteran pests such as corn rootworm (CRW). In addition,

corn 59122 produces the PAT protein from *Streptomyces viridochromogenes* (PAT), which confers tolerance to glufosinate-ammonium, the active ingredient in Liberty herbicide.

Safety of the Expressed Proteins

The safety of Cry1A.105, Cry2Ab2, Cry1F, Cry3Bb1, Cry34Ab1, Cry35Ab1, CP4 EPSPS and PAT proteins has been assessed and approved during safety assessment of MON89034, TC1507, MON88017 and DAS59122-7. The safety assessments showed that the Bt Cry, PAT and CP4 EPSPS proteins do not produce any toxicity at maximum achievable dose levels.

Based on the modes of action and location of these proteins' accumulation in the plant cells, any likelihood of interaction with one another is not considered to be significant to affect the stability and expression level of each gene nor could their interaction lead to production of a new allergen or toxin. Furthermore, there is no known mechanism of interaction among these proteins that could lead to adverse effects in humans or animals.

No unanticipated changes have occurred by combining these events through conventional breeding was demonstrated by bioefficacy assays with MON89034 x TC1507 x MON88017 x Corn 59122. The results of the bioefficacy studies demonstrate that the performance of lepidopteran protection in MON89034 x TC1507 x MON88017 x Corn 59122 is comparable to the same trait in MON89034 and TC1507, as expected. Also it showed that combining MON 89034, Corn 1507, MON 88017, and Corn 59122 by conventional breeding does not alter the tolerance to glyphosate in the combined trait product.

IV. Nutritional Composition (Compositional Analysis)

Compositional and nutritional equivalence of each individual event to conventional corn had been assessed and confirmed by the safety assessment of MON89034, TC1507, MON88017 and DAS59122-7. The World Health Organization (1995) stated that when plants that are substantially equivalent to conventional varieties are crossed by conventional breeding techniques, the combined trait product is expected to be substantially equivalent to the single event products.

V. Anti-Nutritional Factors

A comprehensive evaluation of anti-nutrient components and key secondary metabolites had demonstrated the compositional equivalence of the individual event and conventional corn during the safety assessment of individual event. As described above, the combined trait product by conventional breeding is expected to be substantially equivalent to the single event products,

VI. Regulatory Decision

After reviewing the scientific data and information relevant to the combined traits MON89034 x TC1507 x MON88017 x DAS59122-7 maize application of Monsanto Philippines Inc. and Dow AgroSciences B.V. Philippines, it is concluded that no interaction was found between/among the combined traits, hence this plant product was found to be as safe as its conventional corn and can substitute for its traditional counterpart for direct use as food, feed or for processing. Monsanto Philippines, Inc. and Dow AgroSciences B.V. Philippines are hereby notified that it may

proceed with the activities for the above product for direct use as food and feed or for processing following all existing rules and regulations consistent with DA AO #8 and Memorandum Circular No. 8, Series of 2004.
