

Determination of the Safety of Monsanto's
Corn MON 89034
for Direct use as Food, Feed, and for Processing and for Propagation

Food and Feed and Environmental Safety

The product dossier on Corn MON 89034 was reviewed for safety and nutritional differences compared with the conventional corn. The focus of the review was on any new or altered expression trait and changes in composition and nutritional content or value relative to the conventional corn. At the end of the safety assessment, a conclusion was made that the corn MON 89034 is as safe as the conventional corn taking into account dietary impact of any changes in nutritional content or value and Corn MON 89034 is safe to humans, animals and non-target organisms and as nutritious as ordinary corn.

Biosafety permits for Corn MON 89034 and all progenies derived from crosses of the product with any conventionally-bred corn and corn containing approved-biotech events for direct use as food, feed or for processing and for propagation were issued to Monsanto Philippines Inc. on April 29, 2009 and November 19, 2010 respectively. The permits for direct use and for propagation are valid for five years and shall expire on April 28, 2014 and November 18, 2015 respectively, subject to the terms and conditions set forth in DA Administrative Order No. 8, Series of 2002. The said Corn MON 89034 was included in the Lists of Approval Registry prepared by the Department of Agriculture- Bureau of Plant Industry.

This approval is for use as food, feed and for processing and for cultivation of insect resistant Corn MON 89034 in the Philippines. Food and feed use and Cultivation of Corn MON 89034 and its by-products is therefore authorized as of April 29, 2009 and November 19, 2010_ respectively. The biosafety permit (No. 09-046) for direct use and biosafety permit (No.10-0008) for propagation stated that "Corn MON 89034 is as safe for human food, livestock feed and for processing and propagation as its conventional counterparts" respectively.

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

Designation: Lepidopteran-protected Corn (MON 89034)

Applicant: **MONSANTO PHILIPPINES, INC.**
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Madrigal Business Park
Alabang, Muntinlupa City 1770

Plant Species:

Name: Corn (*Zea mays*)

Parent Material: MON 89034 is the proprietary inbred line, LH172 (Eggerling, 1994). LH172 is a commercial corn line produced by Holdens Company, a Monsanto affiliate.

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 a pediatric allergy specialty center for grain allergy)

Trait Description: Resistant to lepidopteran insect pests

Trait Introduction Method: *Agrobacterium*-mediated transformation

Donor Organisms: *Bacillus thuringiensis* - a source of (Bt) *cry1A.105* and *cry2Ab2* genes

Pathogenicity:

The donor organism for the Cry1A.105 and Cry2Ab2 proteins, *Bacillus thuringiensis* 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.

The characterization of the Cry1A.105 and Cry2Ab2 proteins produced in MON 89034 confirmed that these proteins are equivalent to the respective *Escherichia coli* produced protein standards used in safety studies.

The assessment of potential allergenicity and toxicity showed there was a reasonable certainty of no harm to mammals from exposure to the Cry1A.105 and Cry2Ab2 proteins. These assessments were based on (a) an evaluation of potential allergenicity based on the source of protein, structural to known allergens, in vitro digestibility in simulated digestive fluids and expected dietary exposure and b) an evaluation of potential toxicity based on history of use, similarity to known toxins or biologically active proteins, and evaluation of acute toxicity to mammals

Proposed Use:

For propagation

II. Background Information

Monsanto Philippines, has filed an application with attached technical dossiers to the Bureau of Plant Industry on April 5, 2010 for a biosafety permit for propagation under Administrative Order (AO) No. 8 Part 5 for Corn MON 89034, a second generation product, which has been genetically modified for insect resistance.

The petitioner/applicant published the said application on two widely circulated newspapers: Malaya Business Insights on 17 June 2010 and The Daily Tribune on 17 June 2010 for public comment/review. During the 30-day comment period, BPI had not received comment on the said application.

III. Description of Novel (Introduced) Traits

Corn MON 89034 has been evaluated according to BPI's safety assessment by concerned agencies [Bureau of Animal Industry (BAI), Bureau of Agriculture, Fisheries and Product Standards (BAFPS) and a Scientific and Technical Review Panel (STRP)]. The process involves an intensive analysis of the nature of the genetic modification together with the consideration of safety assessment paradigm which includes molecular characterization, protein characterization, and food/feed composition.

Monsanto has developed a biotechnology derived product corn MON 89034 through *Agrobacterium* mediated transformation to express the *Bacillus thuringiensis* insecticidal proteins Cry1A.105 and Cry2Ab2 using the plasmid vector PV-ZMIR245, which is a binary vector containing two separate transfer DNA's (2T-DNA). The first T-DNA, designated as T-DNA I, contains the *cry1A.105* and the *cry2Ab2* expression cassettes. The second T-DNA designated as T-DNA II contains the *nptII* (neomycin phosphotransferase II) expression cassette, as a selectable marker. Traditional breeding was used to isolate those plants only contained the *cry1A.105* and *cry2Ab2* expression cassettes (T-DNA I) and did not contain the *nptII* expression cassette (T-DNA II), thereby, producing marker-free corn MON 89034. The introduction of the second generation product MON 89034 is expected to provide enhanced benefits for the control of lepidopteran

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insects pests such as *Ostrinia furnacalis* (Asian corn borer, ACB), *Spodoptera litura* (CCW) and *Helicoverpa zea* (corn earworm, CEW) compared to existing products.

Safety of the Expressed Proteins

The donor organism for the Cry1A.105 and Cry2Ab2 proteins, *Bacillus thuringiensis* (*Bt*) is found naturally in soil. *Bt* strains have been used commercially in the US since 1958 to produce microbial-derived products with insecticidal activities. The extremely low mammalian toxicity of *Bt* based insecticide products has been demonstrated in numerous safety studies and there are no confirmed cases of allergic reactions to Cry proteins in applicators of microbial-derived *Bt* products during the past 45 years of use. There are no known reports of allergy or toxicity to *Bt* or to these cry proteins.

Cry1A.105 is a chimeric protein comprised of domains I and II from Cry1Ab and Cry1Ac, domain III from Cry1F (*Bt* subsp. *aizawai*) and the C-terminal protein from Cry1Ac (*Bt* subsp. *kurstaki*). The Cry1A.105 protein produced in MON 89034 is structurally and functionally similar to Cry1A proteins produced in a number of biotechnology-derived crops (Yieldgard corn borer corn, Bollgard cotton and Bollgard II cotton) that have demonstrated safe use.

The Cry2Ab2 protein produced in MON 89034 is derived from the *Bt* species *kurstaki* and its amino acid sequence differs from that of the wild type protein by a single amino acid. The Cry2Ab2 protein has 88% amino acid identity to the Cry2Ab2 protein which is present in commercial microbial products such as Dipel® and Crymax®. The Cry2Ab2 protein produced in corn MON 89034 and Bollgard II cotton have identical amino acid sequence. Bollgard II cotton has been in the market since 2003 and there have been no adverse reports regarding its safety.

The results of the molecular characterization of the inserted DNA confirm the insertion of a single functional copy of the *cry1A.105* and *cry2Ab2* expression cassettes at a single locus within the genome. The data on detailed biochemical characterization of the Cry1A.105 and Cry2Ab2 proteins demonstrate that the two Cry proteins produced in MON 89034 are equivalent to their counterparts produced by recombinant strains of *Escherichia coli* in various safety assessment studies. A thorough assessment of the allergenicity and toxicity potential of the Cry1A.105 and Cry2Ab2 proteins based on extensive studies demonstrate the safety of the two proteins due to the lack of allergenic potential and the lack of acute toxicity when ingested, and their similarity to Cry proteins that have a history of safe use. The results on the estimation of protein levels, and a dietary safety assessment based on anticipated exposure and the results of acute toxicology tests show that there are no meaningful risk to human or animal health from dietary exposure to the two proteins based on the large margins of exposure (MOE) obtained.

Mice acute oral toxicity studies demonstrated that the two proteins: Cry1A.105 and Cry2Ab2, are not acutely toxic and do not cause any adverse effects even at the highest dose level test, which are 2072 and 2198 mg/kg body weight for Cry1A.105 and Cry2Ab2 proteins. Cry1A.105 is structurally and functionally similar to Cry proteins in current commercial products. Cry2Ab2 is the same protein in commercial Bollgard II cotton. Cry1A.105 and Cry2Ab2 proteins, with greater than 95% to 99% of the proteins, respectively are rapidly digestible in simulated gastric fluids (SGF), being digested in less than 30 seconds. Proteins that are rapidly digestible in mammalian gastrointestinal systems are unlikely to be allergens and toxins when consumed. Cry1A.105 and Cry2Ab2 proteins do not share any amino acid sequence similarities with known allergens, gliadins, glutenins, or proteins toxins which have adverse effects to mammals.

IV. Nutritional Composition (Compositional Analysis)

Compositional assessment of the grain and forage from MON 89034 demonstrated that it is nutritionally and biologically equivalent to its conventional counterpart. Compositional data on key nutrients (including proximates, amino acid and fatty acid composition), anti-nutrient and other components collected for the forage and grain from MON 89034 are almost the same or equivalent to those of the conventional control corn grown at five field sites in the US during 2004. The levels of proximates (ash, fat, protein and moisture), fiber (acid detergent fiber-ADF and neutral detergent fiber-NDF), minerals (calcium and phosphorous), and carbohydrates by calculation were compared in forage from MON 89034 and the non-transgenic control. No statistically significant differences were found in the levels of all analyzed components between forage from MON 89034 and forage from the conventional control, with the exception of phosphorus. MON 89034 had statistically significantly higher levels of phosphorus than its

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control, but these levels were within the 99% tolerance intervals for the commercial varieties and within the ranges reported in the literature and the ILSI Crop Composition Database.

The levels of proximates, fiber, minerals, amino acids, fatty acids, carbohydrates by calculation, anti-nutrients, secondary metabolites and vitamins were compared in mature grain from MON 89034 and control maize. A statistical analysis of the analytical results combined from all field trials found no statistically significant differences between MON 89034 and the conventional non-transgenic control, except for stearic acid and arachidic acid (calculated as a percentage of total fatty acids). However, differences in the levels of these fatty acids were small. The levels of all measured components were within the ranges reported in the literature and the ILSI Crop Composition Database and within the 99% tolerance interval established from the 15 conventional, commercial corn hybrids.

The nutritional equivalence of MON 89034 to conventional corn was further confirmed in feeding studies with broiler chicken.

V. Anti-Nutritional Factors

Anti-nutrients in corn include phytic acid, 2,4-dihydroxy-7-methoxy-2H-1,4-benzo-xazin-3(4H)-one (DIMBOA), raffinose, and trypsin and chymotrypsin inhibitors. OECD does not recommend analysis of DIMBOA due to the variable levels found across corn hybrids. Similarly, analysis of trypsin and chymotrypsin inhibitors is not recommended because they occur at low levels in corn and are not considered nutritionally significant. The anti-nutrients phytic acids and raffinose, and the secondary metabolites furfural, ferulic acid and *p*-coumaric acids were analyzed in the grain of MON 89304. There were no statistical differences observed.

VI. Environmental Assessment

The environmental impact of corn MON 89034 is also comparable to conventional corn. The extensive evaluation of the phenotypic and agronomic characteristics and ecological interactions of Corn MON 89034 showed that it is not likely to pose an increased plant pest potential compared to conventional corn and also unlikely to have adverse effects on non-target organisms under the conditions of use.

No change in cultivation practices except the potential to reduce insecticide application against Asian Corn Borer (ACB), corn earworm, and common cutworm. Reduction in insecticide usage will not cause adverse environmental impact. The multi-location field trial showed that the pests and diseases affect both the Bt corn plant and its conventional isohybrids except for Asian corn Borer.

Based from the studies on non-target organisms (NTOs) on MON 89034 and MON 89034 x NK603 (Lit, et al, 2009), overall abundance (based on counts), species diversity and guild structure for both the canopy dwelling NTO and ground dwelling and canopy foraging NTO insects in regulated field trials sites in Luzon and Mindanao specifically in Sta. Maria, Pangasinan, Ilagan, Isabela and Tupi, South Cotabato did not differ significantly across treatment and sites. Abundance of ground dwellers was insignificant across sites and sampling dates. The abundance of parasitoids was not significant across treatments, sites and corn growth stages. The results showed that there were no clear statistically significant differences obtained among treatments across sites suggesting that Corn MON 89034 has no significant positive or negative effects on other herbivores arthropods, predators, parasites or parasitoids in the corn agroecosystems in both the dry and wet seasons.

Field studies conducted over the past decade by industry and the academic community on registered insect-protected crops that produce a variety of Cry proteins have demonstrated that these crops have no adverse effects on the diversity and abundance of non-target arthropod communities including predators, parasitoids and other ecologically important non-target demonstrated that these crops have no adverse effects on the diversity and abundance of non-target arthropod communities including predators, parasitoids and other ecologically important non-target arthropods.¹ Additionally, Cry1 and Cry proteins have been shown to pose low risk to populations of non-target lepidopterans.²

¹ Bitzer et al., 2005; Bhatti et al., 2005; Daly and Buntin, 2005; Dively, 2005; Head et al., 2005; Lopez et al., 2005; Pilcher et al., 2005; Torres and Ruberson, 2005; Whitehouse et al., 2005; Naranjo et al., 2005; Naranjo, 2005a; Naranjo, 2005b; Lozzia et al., 1998; Orr and Landis, 1997; Pilcher et al., 1997.

² Hellmich et al., 2001; Mattila et al., 2005; Pleasants et al., 2001.

The evaluation of the potential toxicity to selected NTOs (hazard assessment) was also conducted. The detailed hazard assessment included toxicity testing against one mammalian species (mice), two avian species (broiler chicken and bobwhite quail), one aquatic species (*Daphnia*), two species of soil decomposers (Collembola and earworm), and four beneficial insect species: honeybee (*Apis mellifera*); minute pirate bugs (*Orius insidiosus*), ladybird beetle (*Coleomegilla maculate*); and parasitic wasp (*Ichneumon promissorius*). These data, combined with the specificity of Bt proteins, and the absence of adverse ecological effects support the conclusion that Bt proteins are unlikely to have an adverse impact on soil organisms.

VII. Regulatory Decision

After reviewing the scientific data and information relevant to the application of Monsanto Philippines Inc., it is concluded that Corn MON 89034 and all progenies derived from crosses of this product with any conventionally-bred corn except when such cross involves another transformation event is as safe and substantially equivalent to its unmodified counterpart, and is therefore approved for propagation.

Monsanto must comply with approved stewardship program consisting of Enhanced Insect Resistance Management (IRM) Strategy, a valid Plant Incorporated Protectant (PIP) permit, other requirements of the Fertilizer and Pesticide Authority, other approved monitoring activities, regular report of seed sales and conduct of required technical studies.