

Determination of the Safety of Monsanto's
Combined Trait Product of Cotton: MON 15985 x MON 1445
for direct use as food, feed and for processing

Food and Feed Safety

The product dossiers on Monsanto's combined trait product Bollgard Cotton (MON 15985) x Roundup Ready Cotton (MON 1445) were reviewed for safety and nutritional differences compared with the conventional cotton. The focus of the food/feed safety assessment is 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.

A biosafety notification for combined trait product Bollgard cotton (event 15985) x Roundup Ready Cotton (event 1445) and all progenies derived from crosses of the product with any conventionally-bred cotton and cotton containing approved-biotech events for direct use as food, feed or for processing was issued to Monsanto Philippines Inc. on November 22, 2004. The notification is valid for five years and shall expire on November 21, 2009 subject to the terms and conditions set forth in DA Administrative order No. 8, Series of 2002, and Memorandum Circulars Nos. 6 and 8, Series of 2004. The said combined trait product was included in the Lists of Approval Registry (Delisting) being prepared by the Department of Agriculture-Bureau of Plant Industry.

This approval is for use as food, feed and processing only. This does not include cultivation of combined trait product Bollgard cotton (MON 15985) x Roundup Ready Cotton (MON 1445) in the Philippines. Food and feed use of combined trait product Bollgard cotton (MON 15985) x Roundup Ready Cotton (MON 1445) and its by-products is therefore authorized as of November 22, 2004. The biosafety notification (No. 04-006) stated that combined trait product Bollgard cotton (MON 15985) x Roundup Ready Cotton (MON1445) is as safe for human food, livestock feed and for processing as its conventional counterparts".

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

Designation:	Combined Trait Product Cotton MON 15985 x MON 1445
Applicant:	MONSANTO PHILIPPINES, INC. 7 th Floor, Ayala Life-FGU Center Alabang-Zapote Road cor Acacia Avenue Madrigal Business Park Alabang 1770, Muntinlupa City
Plant Species:	
Name:	Cotton (<i>Gossypium hirsutum</i> L.)
Parent Material:	Cotton variety Coker 312 and DP50B- Commercial cotton variety of USA.
Center of Origin:	The most important agricultural cottons are <i>G. hirsutum</i> and <i>G. barbadense</i> . These are both allotetraploids of New World origin and presumably of ancient cross between Old World A Genomes

and New World D genomes. Wild diploid species occur in Australia, the Afro-Arabia and America. Wild tetraploid species occur in the New World (Hawaii, Northeastern Brazil, Galapagos, Mexico, Antilles and certain Pacific Islands, South and Central America, coast of Peru, Ecuador and Galapagos Island, Middle America, West Indies, Polynesia, North Africa, tip of Florida). The wild populations of *G. hirsutum* are relatively rare and tend to be widely dispersed.

Toxic Factors/Allergen(s): No known toxicants

Trait Description: Insect resistance and herbicide tolerance

Trait Introduction Method: Conventional breeding

Donor Organisms: *Bacillus thuringiensis* subsp. *kurstaki*, source of *cry2Ab2* and *cryIAc* coding sequences which encode proteins that confer resistance to Lepidopteran insect pest.

Escherichia coli strain K12, source of selectable marker *uidA* gene which encodes the β -D glucuronidase (GUS) protein.

Agrobacterium sp. Strain CP4, the source of *cp4 epsps* gene, confers tolerance to herbicide (glyphosate). Transposon Tn5 from *Escherichia.coli*, the source of the *nptII* gene, confers resistance to the antibiotic kanamycin and was used as a selectable marker.

Pathogenicity: *Bacillus thuringiensis* subsp. *kurstaki* has been shown to be non-toxic to humans, other vertebrates and beneficial insects. *B.t.k.* based foliar insecticides have been registered for over 30 years and have a long history of safe use.

Escherichia coli is a gram-negative, non-pathogenic bacterium used for DNA cloning and vector construction. *Escherichia coli* is ubiquitous in the digestive system of vertebrates, including humans. GUS activity has been detected in a large number of bacteria, mammals and plants including food plants such as potato and apple and constitutes a history of safe exposure.

Agrobacterium sp. strain CP4, is ubiquitous in nature and are not considered to be pathogenic (U.S. FDA, 1994; Harrison, et al., 1996).

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

II. Background Information

In general, to produce products with stacked traits, the process follows a typical backcrossing program as employed in most seed companies. The purpose of backcrossing is to introduce each transgene into a background containing other desirable traits but lacking the target transgene. This typically takes from 5-6 generations to result in the progeny species containing the target gene in the background of the recurrent parent.

Monsanto Philippines, Inc. has filed an application with attached technical dossiers to the Bureau of Plant Industry on September 3, 2004 for a biosafety notification for direct use as food, feed and for processing under Administrative Order (AO) No. 8 Part 5 for combined trait product cotton: MON 15985 x MON 1445 which has been genetically modified for insect resistance and herbicide tolerance.

A safety assessment of combined trait product cotton: MON 15985 x MON 1445 was conducted as per Department of Agriculture Administrative Order No. 8 Series of 2002 and Memorandum Circulars Nos. 6 and 8, Series of 2004. The focus of risk assessment is the gene interactions between the two transgenes.

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 (Introduced) Traits

The *cryIAc* gene and the *cry2Ab* gene derived from *Bacillus thuringiensis* subsp. *kurstaki* confers resistance to lepidopteran pests. The *cp4 epsps* gene encoding 5-enolpyruvylshikimate-3-phosphate synthase confers tolerance to the herbicide glyphosate. The neomycin phosphotransferase II (*npt II*) gene confers resistance to the antibiotic kanamycin and was used as a selectable marker.

A commercial variety with the introduced trait MON 1445 was developed by the traditional backcrossing of MON 1445 and the conventional variety. The resulting variety with MON 1445 was then crossed with another cotton line that contains MON 15985. The resulting variety with the 1445 event was then crossed with another cotton MON 15985 event. A minimum of five to six backcrosses was made to stabilize the introduced MON 15985 with the commercial variety containing MON 1445. The resulting seeds are stacked genes F1 hybrid (MON 15985 x MON 1445).

Safety of the Expressed Proteins

Without stacking, the insertion of *CP4 EPSPS*, *Cry2Ab2* and *CryIAC* in cotton did not result in the occurrence of a new allergen or a new toxin. Since these proteins have not shown to produce any toxicity at maximum achievable dose levels, it is highly unlikely that there will be any interactions between these proteins at normal dosing levels leading to either additive or synergistic effects. There exists an extensive body of literature on the toxicology of the mixtures of chemicals demonstrating that those interactions do not exist when substances are administered at dose levels well below the No Observed Adverse Effect Level (NOAEL).

IV. Nutritional Composition (Compositional Analysis)

Compositional comparison of cottonseed from transgenic lines was made to commercial non-transgenic cottonseed. The compositional analyses of cottonseed included proximates (crude protein,

crude fat, crude fibre, ash and gross energy), amino acid composition, fatty acids profile, aflatoxins and levels of tocopherols.

The concentrations of protein, oil, carbohydrate and ash were the same for transgenic cotton the control. Fatty acid concentration was within the normal published range for cottonseed. Additional analyses of composite samples of cottonseed products (raw meal, toasted meal, kernel, refined oil) showed that the products from the transgenic cotton lines were similar in composition to the control line. Feed studies of a four week rat feeding trial showed no difference in weight gain of animals fed diets containing 10% raw cottonseed meal.

The analysis of the fatty acid composition of refined oil from transgenic cotton did not reveal any significant differences with the parent, non-transgenic variety and was within the normal range reported for cottonseed oils. In addition, the levels of alpha-tocopherol in refined oil from transgenic and control lines were similar. The consumption of refined oil from transgenic cottonseed will have no significant impact on the nutritional quality of the food supply.

V. Anti-Nutritional Factors

Antinutrients, such as cyclopropenoid fatty acids, gossypol, a known toxicant and aflatoxin are known to be present in small amounts in cotton event 15985. These limit their use as protein supplement in animal feed except for cattle which is unaffected by these components. These levels of these antinutrients are compositionally equivalent to conventional cotton. They were within the nontransgenic reference range. Gossypol and mycotoxin content of cotton event 15985 was comparable to the traditional cotton variety. Bollgard II has similar performance as non-transgenic cotton seed based on the feeding studies done on dairy cows.

The food products derived from cotton are cottonseed, cottonseed oil and linters and these food products do not contain detectable levels of protein. Cottonseed oil is commonly used in salad dressings and linters are used in sausage casings. Cottonseed is used as a feed product either in the raw seed form or the defatted meal.

Antinutrients, such as cyclopropenoid fatty acids, gossypol, a known toxicant and aflatoxin are known to be present in small amounts in cotton. These limit their use as protein supplement in animal feed except for cattle which is unaffected by these components. Inactivation or removal of these components during processing enables the use of some cottonseed meal for catfish, poultry and swine. The levels of these components in Roundup Ready Cotton are comparable to the levels of conventional cotton. Gossypol and mycotoxin content of cotton event 1445 was comparable to the traditional cotton variety. RR Cotton has similar performance as non-transgenic cotton seed based on the feeding studies done on dairy cows.

VI. Regulatory Decision

After reviewing the scientific data and information relevant to the combined trait cotton 531 x 1445 application of Monsanto Philippines Inc. it is concluded that no interaction found between/among the combined traits, hence this plant product was found to be as safe as its conventional cotton and can substitute for its traditional counterpart for direct use as food, feed and for processing and is therefore approved for direct use as food, or feed or for processing. Monsanto is 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.