

Determination of the Safety of Monsanto's
Combined Trait Product Cotton: MON 531 x MON 1445
for Direct Use as Food, Feed and for Processing

Food and Feed Safety:

The product dossiers on Cotton event 531 x 1445 were reviewed for safety and nutritional differences compared with the conventional cotton. 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 cotton. At the end of the safety assessment, a conclusion was made that the Cotton event 531 x 1445 is as safe as the conventional cotton taking into account dietary impact of any changes in nutritional content or value.

A biosafety notification for combined trait product cotton: MON 531 x MON 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 cotton MON 531 x MON 1445 in the Philippines. Food and Feed use of combined trait product cotton MON 531 x MON 1445 and its by-products is therefore authorized as of November 22, 2004. The Biosafety notification (No. 04-005) stated that "insect-resistant, herbicide-tolerant cotton combined trait product 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 531 x MON 1445

Applicant: Monsanto Philippines, Inc.
7th Floor, Ayala-FGU Center
Alabang-Zapote Road cor
Acacia Avenue, Madrigal Business Park
Alabang 1770 Muntinlupa City

Plant Species:
Name: Cotton (*Gossypium hirsutum* L.)

Parent Material: Cotton variety Coker 312

Center of Origin: The most important agricultural cottons are *G. hirsutum* and *G. barbadense*. 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 Organism: *Bacillus thuringiensis* subsp. *kurstaki*, source of *cry IAc* gene which confers resistance to lepidopteran insect pest.

Agrobacterium sp. Strain CP4, the source of the *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.

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

Monsanto Philippines, 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 531 x MON 1445 which has been genetically modified for insect resistance and herbicide tolerance.

A safety assessment of combined trait product cotton: MON 531 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

Cotton 531 x Cotton 1445 includes two incorporated genes, *cryIAc* and *cp4epsps* that encode proteins that convey protection from lepidopteran insect pests and tolerance to Roundup herbicide, respectively. The CryIAc protein is an insect control protein and acts through a toxic action in the gut of specific lepidopteran insects. The *cryIAc* gene was derived from the common soil bacterium

Bacillus thuringiensis subsp. *kurstaki*, and was introduced in the Bollgard cotton event 531. CP4 EPSPS protein belongs to the family of EPSP synthases, which are enzymes involved in the penultimate step on the biochemical shikimate pathway producing aromatic amino acids in the chloroplasts of plants. The *cp4 epsps* gene inherited by Bollgard x Roundup Ready from the Roundup Ready cotton event 1445 as derived from *Agrobacterium* sp. Strain CP4, a common soil-borne bacterium.

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 531. The resulting variety with the 1445 event was then crossed with another cotton MON 531. A minimum of five to six backcrosses was made to stabilize the introduced MON 531 with the commercial variety containing MON 1445. The resulting seeds are stacked genes F1 hybrid (*Bollgard x Roundup Ready* cotton).

Safety of Expressed Proteins

Since the proteins produced by the two genes (*cryIac* and *cp4 epsps*) in the stacked cotton variety are products of unrelated biochemical pathways, there will be no interaction effect as to produce a more potent allergen to animals and humans.

The mode of action of each gene product is different. The *cryIac* gene encodes the production of the endotoxin that is toxic to Lepidopterous insects while the *cp4 epsps* gene neutralizes the presence of glyphosate (as sprayed herbicide) and allows the normal production of aromatic amino acids phenylalanine, tyrosine and tryptophan, which are essential in protein synthesis.

The products are involved in different metabolic pathways. There are no possible unexpected effects of the stacked genes on the plant metabolism. Both the CP4 EPSPS and CryIac proteins were considered to have no effect on normal plant metabolism when safety assessment was conducted for each event, and the individual approvals were granted. Expression of Roundup Ready and Bollgard cotton traits in the stacked varieties is not expected to produce interactive or synergistic effects on plant metabolism because different modes of action and binding sites are involved.

The *nptII* gene was used as the selectable marker in the creation of Bollgard and is one of the inserted genes into Bollgard. Thus, when Bollgard was conventionally bred with cotton event 1445, it forms part of the stacks in the individual event

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

Cotton is known for the production of anti-nutritional factors and untreated raw seed is unsuitable as livestock feed for monogastric animals. The transgenic and parental lines were assayed for the presence of potential toxins, including gossypol, dihydrosterculic acid, sterculic acid, malvalic acid and aflatoxins B1, B2, G1 and G2. At detection thresholds of 0.002% or 1 ppb, respectively, neither free gossypol nor any of the four aflatoxins were detected in the oil from transgenic cottonseed. Similarly, the respective levels of the cyclopropenoid fatty acids (dihydrosterculic, sterculic and malvalic) were statistically identical in cottonseed samples from transgenic and control lines

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.