Determination of the Safety of Monsanto’s
Cotton MON 1445 (Glyphosate-Tolerant Cotton)
for Direct Use as Food, Feed and For Processing

Food and Feed Safety:

The product dossier on Cotton MON 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 / nutritional content or value relative to the conventional cotton. At the end of the safety assessment, a conclusion was made that the Cotton MON 1445 is as safe as the conventional cotton taking into account dietary impact of any changes in nutritional content or value.

A biosafety permit for Cotton 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 and for processing was issued to Monsanto Philippines, Inc. on December 5, 2003. The permit is valid for five years and shall expire on December 4, 2008 subject to the terms and conditions set forth in DA Administrative Order No. 8, Series of 2002. The said Cotton MON 1445 was included in the Lists of Approval Registry (Delisting) prepared by the Department of Agriculture-Bureau of Plant Industry.

This approval is for Direct use as food, feed and for processing only. This does not include cultivation of Glyphosate-Tolerant Cotton in the Philippines. Food and Feed use Cotton MON 1445 and its by-products is therefore authorized as of December 5, 2003. The Biosafety Permit (No. 03-012) stated that “Glyphosate-Tolerant Cotton MON 1445 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: Cotton MON 1445 (Roundup Ready™ Cotton)

Applicant: MONSANTO PHILIPPINES, INC.
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Alabang 1770 Muntinlupa City
Philippines

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

Parent Material: Coker 312 Cotton

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): Cotton oil is used for human consumption and there is very little protein in the oil, cottonseed oil is not a potential source of allergens. Aflatoxins and gossypol are present in the cotton seeds. Gossypol is naturally occurring in the cottonseed while aflatoxin is a fungal secondary metabolite which accumulates during fungal infection.

Trait Description: Herbicide tolerance

Trait Introduction Method: Agrobacterium-mediated transformation

Donor Organism: Agrobacterium spp. Strain CP4, source of the source of the cp4 epsps gene, confers tolerance to the herbicide glyphosate. Transposons (Tn5 and Tn7) from Escherichia coli, source of nptII gene, encodes a selectable marker enzyme, neomycin phosphotransferase II (NPTII), which was used to identify transformed cotton cells containing the CP4 EPSPS protein.

Pathogenicity: There have been no reports of adverse effects on Agrobacterium sp., a naturally occurring bacterium encoding for a CP4EPSPS protein on human and animals. CP4 EPSPS, being not from a known allergenic source, has a long history of safe use in human and animal consumption.

Escherichia coli is a gram-negative, non-pathogenic bacterium used for DNA cloning and vector construction.

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

II. Background Information

Cotton is the leading plant fiber crop produced in the world and is grown primarily for its fiber. Cottonseed is processed to produce animal feed ingredients. Cottonseed meal is primarily used as cattle feed, with smaller proportions of meal fractions used in feed for poultry, sheep, catfish and swine. Cottonseed serves as an excellent source of fiber and protein, particularly due to its high lysine content. Oil is the main food ingredient derived from cottonseed and is used for frying oil and in salad dressings.

Monsanto Philippines Inc. has developed cotton lines, derived from the variety Coker 312, which expresses their proprietary Roundup-Ready™ genes. These confer novel tolerance to glyphosate,
the active ingredient of Roundup® herbicide, which can control or suppress economically important weeds in cotton production

On May 29, 2003, Monsanto Philippines Inc. submitted an application to the Bureau of Plant Industry (BPI) requesting for Biosafety Permit under Administrative Order Number 8 (AO#8) Part 5 for Cotton MON 1445 which has been genetically modified for herbicide resistance.

Monsanto Philippines Inc has provided data on the identity of the glyphosate tolerant cotton lines Cotton MON 1445, a detailed description of the modification method, data and information on the gene insertion sites, copy numbers and levels of expression in the plant, the role of the inserted genes and regulatory sequences in donor organisms, and full nucleotide sequences. The novel proteins were identified, characterized, and compared to the original bacterial proteins, including an evaluation of their potential toxicity to livestock. Relevant scientific publications were also supplied.

Cotton MON 1445 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 Technical Review Panel (STRP). The process involves an intensive analysis of the nature of the genetic modification together with a consideration of general safety issues, toxicological issues and nutritional issues associated with the modified cotton.

The petitioner/applicant published the Public Information Sheet (PIS) of the said application on two widely circulated newspapers: Malaya and Daily Tribune, on June 30, 2003 for public comment/review. BPI received no comment on the petition during the 30-day comment period.

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 MON 1445 (Trade name: Roundup-Ready Cotton) and all cotton lines/hybrids derived from this Event contain the CP4 EPSPS coding sequence from Agrobacterium sp CP4 strain. The CP4 EPSPS sequence encodes for the production of the naturally-occurring CP4 EPSPS protein that renders the Cotton MON 1445 tolerant to glyphosate, a herbicide known to be environmentally-compatible. The EPSPS enzyme is present in the shikimic acid pathway for the biosynthesis of aromatic amino acids in plants and microorganisms. Inhibition of this enzyme by glyphosate leads to a deficiency in the production of aromatic acids and lack growth in plants. The aromatic amino acid biosynthetic pathway is not present in mammalian, avian or aquatic animals. This explains the selective activity in plants and contributes to the low risk to human health and the environment from the use of glyphosate according to label recommendations.

The intended effect of the genetic modification is to make cotton (Gossypium hirsutum L.) plants tolerant to the non-selective herbicide glyphosate. Glyphosate's herbicidal activity is conferred by its ability to potently inhibit the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS). The EPSPS enzyme catalyzes the conversion of shikimate-3-phosphate (S-3-P) and phosphoenolpyruvate (PEP) into 5-enolpyruvylshikimate-3-phosphate (EPSP), an intermediate in the production of aromatic amino acids that takes place in the chloroplast. The EPSPS enzyme from Agrobacterium sp. strain CP-4 (CP4 EPSPS) is highly resistant to inhibition by glyphosate.
Monsanto used the *Agrobacterium* sp. strain CP4 EPSPS gene to confer tolerance to the herbicide in their transgenic cotton.

Monsanto transformed Coker 312 cotton with a disarmed *Agrobacterium tumefaciens* double border plant transformation plasmid to produce their glyphosate tolerant Cotton event 1445. Molecular characterization demonstrates that one T-DNA (transfer DNA) insert was integrated into the cotton genome. The insert contains the CoMVb promoter region, the 5-enolpyruvylshikimate-3-phosphate synthase (*cp4 epsps*) gene from *Agrobacterium* sp. strain CP4; a neomycin phosphotransferase gene (*nptII*) from transposon *Tn5*; a bacterial selectable marker gene, *aad*, which provides resistance to spectinomycin; and a portion of *ori-V* coding regions.

**Safety of the Expressed Proteins**

CP4 EPSPS and NPTII proteins introduced into Cotton MON 1445 have a long safe history of use and do not share biochemical properties common to known allergenic proteins. The two proteins show no significant homology to any of the amino acid sequences reported for allergens in the three current protein data bases (Swissprot, Genepept, and Pir protein).

The CP4 EPSPS protein possesses none of the other characteristics common to protein allergens; such as stability to digestion on acidic condition, heat stability and high concentration in food. CP4 EPSPS protein is rapidly degraded by proteolytic enzymes (digestibility in vitro), limiting the exposure of the GIT and less likelihood that the protein can exert pharmacological, toxic or allergic effects. Oral toxicity studies in animals confirmed the non-toxic nature of CP4 EPSPS.

The NPTII protein was also not heat stable and can be rapidly degraded by proteases present in mammalian intestine. The CP4 EPSPS and NPTII proteins should not pose any allergenic risks from consumption of food, feed and by products derived from Cotton MON 1445.

**IV. Nutritional Composition (Compositional Analysis)**

Under the same agronomic condition as conventional counterpart cotton, the CP4 EPSPS does not alter the quantity and quality of the key components. The nutritional equivalence and wholesomeness of Cotton MON 1445 with regards to the key nutrients including proximates, amino acids and fatty acids composition as well as antinutrients, are comparable to conventional cotton.

The nutritional equivalence of Cotton MON 1445 compared to conventional cotton was confirmed in various feeding studies with catfish and quails which included clinical and histological evaluations. Feeding studies with lactating cows similarly showed that RR Cotton did not affect dry matter intake, milk yield, milk composition and body condition score of dairy cows. Processing is unlikely to alter the compositional components of cotton and therefore, products derived from Roundup Ready Cotton seeds will also be substantially equivalent to and as safe as the current cotton-derived products.

**V. Anti-Nutritional Factors**

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 MON 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 application of Monsanto Philippines Inc., it is concluded that Cotton 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 or feed or for processing is as safe and substantially equivalent to its unmodified counterpart, and is therefore approved for direct use as food, or feed or for processing. Monsanto shall duly inform the public of this approval by 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.