Determination of the Safety of Monsanto's
Alfalfa Events J101 and J163
for Direct use as Food, Feed, or Processing

Food and Feed Safety

The product dossiers on Monsanto's alfalfa events: J101 and J163 were reviewed for safety and nutritional differences compared with the conventional alfalfa. 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 alfalfa. At the end of the safety assessment, a conclusion was made that the alfalfa J101 and J163 is as safe as the conventional alfalfa taking into account the safety and nutritional quality of J101 and J163.

A biosafety permit for Herbicide (Glyphosate) Tolerant alfalfa events J101 and J163, and all progenies derived from crosses of the product with any conventionally bred alfalfa and alfalfa containing approved-biotech events for direct use as food, feed or for processing was issued to Monsanto Philippines, Inc. on August 9, 2006. The biosafety permit is valid for five years and renewed on August 9, 2011 and shall expire on August 8, 2016 subject to the terms and conditions set forth in DA Administrative Order No. 8, Series of 2002, as amended by DA Administrative Order No. 22, Series of 2007. The said products were 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 Processing only. This does not include cultivation of alfalfa events J101 and J163 in the Philippines. Food and Feed use of products alfalfa: J101 and J163, and its by-products are therefore authorized as of August 9, 2006. The biosafety notification (No. 06-024) stated that alfalfa events J101 and J163 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: Alfalfa J101 and J163

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

Alabang, Muntinlupa City, Philippines 1781

Plant Species:

Name: Alfalfa (Medicago sativa)

Parent Material: Proprietary alfalfa clone 'R2336.'

Center of Origin:	Asia Minor, Transcaucasia, Turkemistan and Iran. It is also endemic throughout the Mediterranean region, North Africa, the Middle East, most of Europe, Siberia, Northern India and China (Ivanov, 1988; Michaud et al., 1988; Quiros and Bauchan, 1988).
Toxic Factors/Allergen(s):	Lignin, phytoestrogens.
Trait Description:	Herbicide tolerance
Trait Introduction Method:	Agrobacterium -mediated transformation
Donor Organisms*:*	*Agrobacterium tumefaciens strain CP4,* a source of *cp4 epsps* which provide high level of tolerance to glyphosate
Pathogenicity:	Agrobacterium tumefaciens is not known be toxic, allergenic or specifically pathogenic to humans or animal . The CP4 EPSPS protein it produces is functionally equivalent to native EPSPS except for its glyphosate affinity. The safe use of this protein is demonstrated in the similarity of CP4 EPSPS proteins found in fungal and microbial food sources.
Proposed Use:	For direct use as food, feed or for processing

II. Background Information

Monsanto company Forage Genetics International (FGI) havedeveloped alfalfa lines J101 and J163 with tolerance to the herbicide glyphosate.

On October 4, 2005, Monsanto Philippines, Inc. submitted an application to the Bureau of Plant Industry requesting for biosafety permit under Administrative Order (AO) No.8 for glyphosate tolerant alfalfa lines containing the transformation events J101 and J163 individually.

Monsanto have provided data and/or information on the identity of lines J101 and J163 including a detailed description of the transformation method, the safety of donor organism, the role of the inserted genes and regulatory sequences, the insertion sites, copy number and the genetic stability of the insert(s), and levels of expression in the plant. The introduced protein was identified, characterized and evaluated for their potential toxicity and allergenicity to human and livestock. Relevant scientific publications were supplied.

Alfalfa J101 and J163 have been evaluated according to BPI's safety assessment by concerned agencies: Bureau of Animal Industry (BAI), BPI, Bureau of Agriculture and Fisheries Standards (BAFS) and a Scientific and Technical Review Panel (STRP) members. The process involved an extensive safety evaluation of the nature of the genetic modification with a consideration of general safety issues, toxicological and nutritional issues associated with the alfalfa products.

The Public Information Sheet (PIS) of the said application was published in two widely circulated newspapers: The Manila Times and Malaya on November 18, 2005 for public comments/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 completed the approval process.

III. Description of Novel (Introduced) Traits

Roundup Ready alfalfa events J101 and J163 were produced using Agrobacterium-mediated transformation by incorporating the cp4 epsps gene expression cassette encoding for glyphosate tolerance. The *cp4 epsps* gene expression cassette is fused to a chloroplast transit peptide (CTP2) sequence that targets the CP4 EPSPS protein to the chloroplasts, the site of aromatic amino acid synthesis. The soil bacterium Agrobacterium sp. strain CP4, is the donor of the cp4 epsps gene conferring tolerance to glyphosate herbicide.

Safety of the Expressed Proteins

A history of safe use has been established for J101 and J163 CP4 EPSPS. The CP4 EPSPS protein present in Roundup Ready alfalfa is functionally similar to a diverse set of EPSPS proteins typically present in food and feed derived from plant and microbial sources. The EPSPS proteins are required for the production of aromatic amino acids in plants and microbes. J101 and J163 CP4 EPSPS proteins encoded by the inserted cp4 epsps gene do not interfere with normal functioning of the shikimate acid pathway, where EPSPS lies within, as evidenced by the levels of key downstream metabolites such as aromatic amino acids.

Another aspect used for the assessment of potential toxic effects of proteins introduced into plants is to compare the amino acid sequence of the protein to known toxic proteins. J101 and J163 CP4 EPSPS protein does not show meaningful amino acid sequence similarity when compared to known protein toxins.

In vitro simulated mammalian gastric and intestinal digestive mixtures were used to assess the susceptibility of the CP4 EPSPS protein to proteolytic digestion. Rapid degradation of the protein correlates with limited exposure to the gastrointestinal tract and little likelihood that the protein can produce pharmacological, toxic or allergenic effects. The CP4 EPSPS protein was shown to be rapidly degraded in these *in vitro* studies, greatly minimizing any potential for this protein to be absorbed by the intestinal mucosa.

To confirm the lack of acute toxicity, oral toxicity studies with the CP4 EPSPS protein as the test material was performed on mice to directly assess any potential toxicity associated with the protein. CP4 EPSPS protein did not exhibit any signs of toxicity when administered to mice via oral gavage.

This weight of evidence supported the conclusion that CP4 EPSPS is unlikely to exhibit toxic effects when consumed in food and feed prepared from J101 and J163.

IV. Nutritional Composition (Compositional Analysis)

Alfalfa J101 and J163, and its progenies are not significantly different from conventional alfalfa in terms of compositional, nutritional and safety aspects. Compositional analyses of the forage samples included proximates (protein, fat, ash and moisture), acid detergent fiber (ADF), neutral detergent fiber (NDF), lignin, amino acids, and minerals (calcium, copper, iron, magnesium, manganese, phosphorous, potassium, sodium and zinc), coumestrol, as well as carbohydrates by calculation. Studies have shown that forage produced by alfalfa plants containing J101 or J163 is comparable to forage produced by control or conventional alfalfa varieties and is compositionally equivalent to forage derived from conventional alfalfa varieties currently on the market.

V. Anti-Nutritional Factors

Potential anti-nutritional factors present in alfalfa are lignin and phytoestrogen in the form of coumestrol. The level of lignin in events J101 and J163 are comparable to the lignin levels in conventional alfalfa. Similarly, there were no significant difference in the levels of coumestrol in the forage of alfalfa J101 and J163 compared to that of control alfalfa.

VI. Regulatory Decision

Based on the results of the risk evaluation based on the submitted scientific data and other information relevant to the application of Monsanto Philippines Inc., it is concluded that Alfalfa events J101 and J163, and all progenies derived from crosses of the product with any conventionally-bred alfalfa, and alfalfa containing approved-biotech events for direct use as food, feed or for processing is as safe and substantially equivalent to its unmodified counterpart, and is therefore approved for direct use as food, feed or for processing.

Monsanto Philippines Inc. 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, and that imports of these products are covered by conditions for approval as provided in Department of Agriculture Memorandum Circular No. 8, Series of 2003.