

Determination of the Safety of Monsanto's
Alfalfa (Herbicide Tolerant Alfalfa)
for Direct use as Food, Feed, and for Processing

Food and Feed Safety

The product dossier on 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. Safety assessment includes characterization of introduced proteins, confirmation of their functions and physicochemical properties and confirmation of the safety of each protein produced from the inserted DNA. At the end of the safety assessment, a conclusion was made that Alfalfa Events J101 and J163 are as safe as the conventional alfalfa and shall not pose any significant risk to human and animal health.

A biosafety permit for 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 were issued to Monsanto Philippines., Inc. on August 9, 2006. The permit is valid five years and shall expire on August 8, 2011 subject to the terms and conditions set forth in DA Administrative order No. 8, Series of 2002. The said Alfalfa Events J101 and J163 was included in the Lists of Approval Registry (Delisting) 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 Herbicide tolerant Alfalfa Events J101 and J163 in the Philippines. Food and Feed use of Alfalfa Events J101 and J163 and its by-products is therefore authorized as of August 9, 2006. The Biosafety Permit (No. 06-024) stated that "Herbicide Tolerant Alfalfa" 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: Alfalfa Events J101 and J163

Applicant: **MONSANTO PHILIPPINES, INC.**
7th Floor, Ayala Life-FGU Center
Alabang-Zapote Road cor Acacia Avenue
Madrigal Business Park
Alabang 1770, Muntinlupa City

Plant Species:

Name: Alfalfa (*Medicago sativa* L.)

Parent Material: Alfalfa clone R2336 which was selected from an elite high yielding, fall dormant FG1 alfalfa breeding population.

Center of Origin: Alfalfa originated in Asia Minor, Transcaucasia, Turkemistan and Iran and is 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): There were no toxicants identified. The food uses of alfalfa are extremely minor. Alfalfa is consumed by humans in the form of compressed leaf material for dietary supplements and herbal teas. Additional historical or current food uses for alfalfa are extremely limited. Verified reports of human allergenicity to food products derived from alfalfa are extremely rare.

Trait Description: Herbicide Tolerance

Trait Introduction Method: *Agrobacterium tumefaciens* -mediated transformation

Donor Organisms: *Agrobacterium* spp Strain CP4, a source of *cp4 epsps* which provide high level of tolerance to glyphosate.

Pathogenicity: Safety assessment of CP4 EPSPS protein in J101 and J163 included digestibility in simulated gastric fluids, bioinformatics analysis of the structural similarity to known proteins (including protein toxins), acute oral toxicity of mouse gavage, and an evaluation of the protein to EPSPSs naturally present in foods with a long history of safe use (e.g., soybeans and corn). From these studies, there appears to be no known pathogenicity or allergenicity of the encoded protein.

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

II. Background Information

Alfalfa (*Medicago sativa* L.) is recognized as the oldest plant grown solely for forage. It is highly valued for animal feed because of its high protein content, high intake potential and digestibility. It can provide the sole plant component in many livestock feeding programs when supplemented with the proper minerals.

Monsanto had developed varieties from Roundup Ready alfalfa that contain events J101 and J163 and are tolerant to glyphosate, the active ingredient in Roundup agricultural herbicides. Roundup Ready alfalfa varieties were developed using *Agrobacterium* mediated transformation to stably incorporate into the alfalfa genome a coding sequence that produces a glyphosate-tolerant form of the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS). This glyphosate-tolerant EPSPS was originally identified in the soil microorganism, *Agrobacterium* sp. strain CP4 and is designated as CP4EPSPS.

Monsanto Philippines, Inc. has filed an application with attached technical dossiers to the Bureau of Plant Industry on October 4, 2005 for a biosafety permit for direct use as food, feed and for processing under Administrative Order (AO) No. 8 Part 5 for Alfalfa events J101 and J163 which has been genetically modified for herbicide tolerance.

Monsanto Philippines, Inc. has provided data on the identity of Alfalfa Events J101 and J163, a detailed description of the transformation method, data and information on the gene insertion sites, copy number 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 and non-target organisms. Relevant scientific publications were supplied.

Alfalfa Events J101 and J163 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.

The petitioner/applicant published the said application on two (2) widely circulated newspapers: Malaya and Manila Times on November 18, 2005 for public comment/review. During the 30-day comment period, BPI had not received comment on the said application

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

Monsanto has developed a biotechnology derived product Alfalfa events J101 and J163 through *Agrobacterium tumefaciens*-mediated transformation of the alfalfa cultivar using plasmid vector PV-MSHT4. Plasmid vector PV-MSHT4 contains a gene cassette encoding the CP4EPSPS protein (5-enolpyruvylshikimate 3-phosphate synthase) from *Agrobacterium* sp. Strain CP4 that provides tolerance to glyphosate, the active ingredient in the Roundup family of agricultural herbicides (Padgett *et al.*, 1996)

Safety of the Expressed Proteins

Safety assessment of CP4EPSPS protein in J101 and J163 included digestibility in simulated gastric fluids, bioinformatics analysis of the structural similarity to known proteins (including protein toxins), acute oral toxicity by mouse gavage and an evaluation of the protein to EPSPSs naturally present in foods with a long history of safe use (e.g. soybeans and corn). From these studies there appears to be no known pathogenicity or allergenicity of the encoded protein

IV. Nutritional Composition (Compositional Analysis)

Comparison of the proximate composition of forage from Roundup ready alfalfa Event J101 and non-transgenic control showed no statistically significant differences except cystine, glutamic acid,

tyrosine, ash, iron and lignin but the magnitude of the differences were observed to fall within the 99% tolerance interval which does not suggest biological significance.

Comparison of the proximate composition of forage from Roundup ready alfalfa event J163 and non-transgenic control showed no statistically significant differences except in cystine, histidine, lysine, tyrosine and lignin but the magnitude of the differences were observed to fall within the 99% tolerance interval. These differences were considered to be not biological meaningful.

V. Anti-Nutritional Factors

Lignin, a dietary fiber, is considered to be the key anti-nutritional factor associates with alfalfa. Compositional analysis showed that the level of lignin in events J101 and J163 are comparable to lignin levels in conventional alfalfa, as the level of lignin was found to be within the reported literature range in non-transgenic alfalfa.

Phytoestrogens of the coumestan group and saponins are also found in alfalfa. The insignificant amounts of lignin and coumestrol in Roundup ready alfalfa were comparable with control alfalfa.

VI. Regulatory Decision

After reviewing the scientific data and 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 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 the 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.