

Determination of the Safety of Monsanto's
Corn LY038 (Lysine maize)
for Direct use as Food, Feed and for Processing

Food and Feed Safety:

The product dossier on Corn LY038 (Lysine maize) were reviewed for safety and nutritional differences compared with the conventional corn. 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 corn. At the end of the safety assessment, a conclusion was made that the Corn LY038 (Lysine maize) is as safe as the conventional corn and that it produce its intended nutritional benefit for high lysine content.

A biosafety permit for Corn LY038 and all progenies derived from crosses of the product with any conventionally-bred corn and corn containing approved-biotech events for direct use as food, feed and processing were issued to Monsanto Philippines, Inc. on May 19, 2006. The permit is valid for five years and shall expire on May 18, 2011 subject to the terms and conditions set forth in DA Administrative order No. 8, Series of 2002. The said Corn LY038 was included in the Lists of Approval Registry (Delisting) being 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 Corn LY 038 in the Philippines. Food and Feed use of Corn LY 038 and its by-products is therefore authorized as of April 10, 2006 . The biosafety permit (No. 05-023) stated that "High Lysine Corn LY 038" 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: Corn LY038 (Lysine Maize)

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

Plant Species:

Name: Corn (*Zea mays* L.)

Parent Material: H99 inbred line, Cre-*lox* corn line

Center of Origin: Mexico, Central America, South America

Toxic Factors/Allergen(s): Trypsin inhibitor, phytic acid, and secondary metabolites such as raffinose, ferulic acid and p-coumaric acid are present in low amount 2-4 dihydroxy-7-methoxy-2H-1, 4 benzoxazin- 3(4H)-one (DIMBOA) a potential toxicant but declines rapidly as the plant grows

Trait Description:	High Lysine corn
Trait Introduction Method:	Particle bombardment
Donor Organisms:	<i>Corynebacterium glutamicum</i> , a non-pathogenic species of coryneform bacteria, is a natural producer of glutamic acid. Coryneform bacteria are rod-shaped, fast growing, non-sporulating Gram-positive bacteria that are widely distributed. <i>C. glutamicum</i> is used by the chemical industry for the biotechnological production of the amino acid lysine, an essential amino acid in animal nutrition.
Pathogenicity:	<i>Corynebacterium glutamicum</i> (<i>Brevibacterium flavum</i>) is a common soil bacterium widely distributed in the environment and not an animal or human pathogen. Strains of <i>C. glutamicum</i> have been used for commercial fermentation production of a number of amino acids including L-arginine, L-glutamic acid, L-leucine, L-lysine, L-phenylalanine, L-tryptophan, and L-valine. Commercial lysine production is primarily via fermentation of <i>C. glutamicum</i> strains that express cDHDPS, an enzyme with decreased sensitivity of the cDHDPS enzyme from <i>C. glutamicum</i> feedback inhibition facilitates increased flux through the enzymatic step on the lysine biosynthetic pathway, resulting in accumulation of free lysine. A history of safe use of cDHDPS protein in LY038 to DHDPSs naturally present in feed and food (e.g. corn, rice, soy and wheat).
Proposed Use:	For direct use as food, feed and for processing

II. Background Information

Lysine maize LY038 was developed through the use of recombinant DNA techniques, to stably integrate *cordapA* coding sequence into the corn genome, to increase the level of lysine in grain for animal feed applications.

Development of LY038 provides an alternative to direct addition of supplemental lysine to poultry and swine diets by increasing the amount of lysine in the corn component of feed. Introduction of the *cordapA* gene into the corn genome produces a corn grain with higher lysine content and higher nutritional value for use as a feed ingredient for animals, primarily poultry (broilers and turkeys) and swine. LY038 maize grain will be identity preserved and used as a feed ingredient in poultry and possibly swine diets.

Monsanto Philippines, Inc. submitted an application on April 25, 2005 to the Bureau of Plant Industry requesting for biosafety permit under Administrative Order (AO) No. 8 Part 5 for Corn LY038 which has been genetically modified for high lysine content. Monsanto Philippines, Inc. has provided data on the identity of Corn LY038, 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.

Lysine maize, LY038 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 general safety issues, toxicological issues and nutritional issues associated with modified corn.

The petitioner/applicant published the said application on two widely circulated newspapers, Manila Times and Malaya on March 3, 2006 for public comment/review. During the 30-day comment period, BPI had received comments from Third World Network (TWN) on the comments of New Zealand Institute of Gene Ecology of not recognizing/trusting the MSL-18883 study on compositional analysis of four commercial varieties used and safety assessments of LY038 on broilers. The issues raised were reassessed by the STRP and found that Corn LY038 is safe and the issues raised has no safety concerns. Overall, corn LY038 was found to be essentially equivalent to its conventional counterpart except for its high lysine.

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

Lysine maize LY038 contains the *cordapA* coding sequence isolated from *Corynebacterium glutamicum* that encodes the lysine-insensitive dihydodipicolinate synthase (cDHDPS) enzyme. The transcription of *cordapA* is under the control of the corn Glb1 promoter, which directs cDHDPS expression predominantly in the germ, resulting in accumulation of free lysine in grain. In *cordapA* cassette, the cDHDPS coding sequence is under the control of *Zea mays* globulin 1 (Glb1) promoter, which in wild-type corn directs expression of the most abundant embryo-specific protein in corn. The utilization of the Glb1 promoter for *cordapA* transcription results in the expression of cDHDPS and the accumulation predominantly in the germ portion of the grain. The intron-sequence following Glb1 promoter is derived from the *rice actin-1* gene, and the purpose of the element is to enhance DNA transcription. The 3' nontranslated region of the *globulin 1* gene following the *cordapA* gene contains the polyadenylation signal that directs the termination and maturation of the *cordapA* gene transcript.

Lysine maize was developed through the introduction of DNA into callus tissue from corn inbred line H99 by a particle acceleration methodology. Circular map of the plasmid vector PV-ZMPQ76 was used to generate the 5.9 kb *Xho* I linear fragment for biolistic transformation to produce LY038.

Corn plants expressing *Cre recombinase* were crossed with plants positive for the *cordapA* gene that were regenerated from callus tissue transformed with the 5.9 kb linear DNA fragment.

Lysine maize (LY038) and progenies are approved for food/feed/processing and for environmental release by regulatory agencies in the USA. These agencies, relying on their existing internationally-accepted guidelines, processes and principles developed by their and other international safety experts (e.g., those convened by the FAO-WHO Joint Experts consultations, the OECD, ILSI and others), arrived at the decision that LY 038 and its products have been found to be as safe as its conventional counterpart.

Safety of the Expressed Proteins

Corynebacterium glutamicum (*Brevibacterium flavum*) is a common soil bacterium widely distributed in the environment and not an animal or human pathogen. Strains of *C. glutamicum* have been used for commercial fermentation production of a number of amino acids including L-arginine, L-glutamic acid, L-leucine, L-lysine, L-phenylalanine, L-tryptophan, and L-valine. Commercial lysine production is primarily via fermentation of *C. glutamicum* strains that express cDHDPS, an enzyme with decreased sensitivity of the cDHDPS enzyme from *C. glutamicum* feedback inhibition facilitates increased flux through the enzymatic step on the lysine biosynthetic pathway, resulting in accumulation of free lysine.

A history of safe use of cDHDPS protein in LY038 to DHDPSs naturally present in feed and food (e.g. corn, rice, soy and wheat).

IV. Nutritional Composition (Compositional Analysis)

Lysine maize (LY038) will be utilized as a feed ingredient in poultry and possibly swine diets. Because LY038 is not intended to be used for food, human consumption of the cDHDPS protein from processed grain products is expected to be low. Possible human exposure to cDHDPS from LY038 is further decreased by the fact that expression of the cDHDPS protein in LY038 is primarily in the germ portion of the grain, while the endosperm is the predominant corn fraction consumed by humans from typical processing (wet and dry milling).

All statistical analyses showed that for 94.4% of the 396 comparisons mad between LY038 and the negative segregant control, LY038 (-), with respect to their proximate composition, the values were either not statistically different or they were within the calculated 99% tolerance interval for the population of 20 commercial references. In the case of grain, the statistically significant differences were attributed to the intended higher lysine and the free lysine content, and the resulting catabolites saccharopine and α -amino adipic acid content. The remaining statistical differences were only detected in one or two of the five sites, and test values were within ranges reported historically or in scientific literature, with the exception of Total Dietary Fiber where the differences outside of the content of lysine and its catabolites were not considered biologically relevant.

V. Anti-Nutritional Factors

Levels of antinutrients (phytic acid and raffinose) in LY038 were not found statistically different from negative segregant control LY038(-), and were found within the range of 20 commercial varieties, as well as values reported historically or in the scientific literature.

VI. Regulatory Decision

After reviewing the scientific data and information relevant to the application of Monsanto Philippines Inc., it is concluded that Corn LY038 and all progenies derived from crosses of the product with any conventionally-bred corn, and corn containing approved-biotech events for direct use as food, feed and for processing is as safe and substantially equivalent to its unmodified counterpart except for its high lysine content, 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.