

Determination of the Safety of Monsanto's
New Leaf Plus Potato Events RBMT 21-129, RBMT 21-350 and
RBMT 22-82 (Colorado Potato Beetle and PLRV Resistant Potato)
for Direct use as Food, Feed and for Processing

Food and Feed Safety:

The product dossier on New Leaf Plus Potato events RBMT 21-129, RBMT 21-350 and RBMT 22-82 were reviewed for safety and nutritional differences compared with the conventional potato. The focus of the review was on any new or altered expression of traits and changes in composition and nutritional content or value relative to the conventional potato. At the end of the safety assessment, a conclusion was made that the New Leaf Plus Potato events RBMT 21-129, RBMT 21-350 and RBMT 22-82 and its products have been found to be as safe as the conventional potato taking into account dietary impact of any changes in nutritional content or value.

A biosafety permit for New Leaf Plus Potato events RBMT 21-129, RBMT 21-350 and RBMT 22-82 and all progenies derived from crosses of the product with any conventionally-bred potato and potato containing approved-biotech events for direct use as food, feed and for processing was issued to Monsanto Philippines, Inc. on September 24, 2004. The permit is valid for five years and shall expire on September 23, 2009 subject to the terms and conditions set forth in DA Administrative Order No. 8, Series of 2002. The said New Leaf Plus Potato events RBMT 21-129, RBMT 21-350 and RBMT 22-82 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 insect and virus resistant potato events RBMT 21-129, RBMT 21-350 and RBMT 22-82 in the Philippines. Food, Feed and use of its by-products is therefore authorized as of September 24, 2004. The biosafety permit stated that "insect and virus resistant New Leaf Plus Potato" 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:	New Leaf Plus Potato Events RBMT 21-129, RBMT 21-350 and RBMT 22-82
Applicant:	MONSANTO PHILIPPINES, INC. 7 th Floor, Ayala-FGU Center Alabang-Zapote Rd., cor Acacia Avenue Madrigal Business Park Alabang 1770 Muntinlupa City.
Plant Species:	
Name:	Potato (<i>Solanum tuberosum</i> L.)
Parent Material:	Russet Burbank
Center of Origin:	South America particularly the high plateau region of Bolivia and Peru.
Toxic Factors /Allergen(s):	Toxicants that are naturally occurring in the potato tubers such as glycoalkaloids solanine and chaconine are within the standard levels established previously for potatoes.
Trait Description:	Resistance to the Colorado Potato Beetle and Potato Leafroll Virus
Trait Introduction Method:	<i>Agrobacterium</i> mediated transformation method
Donor Organism:	<i>Agrobacterium</i> sp. strain CP4, the causative agent for crown gall disease, is the source of <i>cp4 epsps</i> gene which confers resistance to glyphosate. <i>Bacillus thuringiensis</i> subsp. <i>tenebrionis</i> (<i>B.t.t</i>) is source of <i>cry3A</i> which produce protein toxins specific to target organisms.

Pathogenicity:

Agrobacterium sp. strain CP4, is ubiquitous in nature and are not considered to be pathogenic (U.S. FDA, 1994; Harrison, *et al.*, 1996).

B. thuringiensis subsp. *tenebrionis* is a common soil bacterium that has a long history of safe use as a microbial insecticide with no reported allergenic and toxic responses, establishing basis for the lack of allergenic or toxic concern for the Cry3A protein. Bt based products have shown that the proteins produce toxic effects only in the gut of chewing insects and are not activated in human digestive tracts. The *cry3A* gene encodes for a protein, normally produced by the bacterium during sporulation, which is toxic only to targeted leaf feeding beetles.

Proposed Use:

For direct use as food, feed or for processing

II. Background Information

Potato is grown in over 120 countries primarily for human consumption in its processed form. For direct consumption it is cooked before eaten and its processed form are potato fries, crisps and dehydrated potatoes. It is also used as raw material for starch manufacturing. The starch can be used for coating of papers, sizing of cotton and as finishing in textile industry. By-products (pulp, coagulated protein from fruit water) are normally used in animal feeding, but trends exist for food use too. Potatoes are also used for industrial alcohol production. In countries with a significant potato processing industry (for both human food and industrial use), the residues and by-products (peel, trimmings, rejected potatoes, separated pulp and proteins) are used as feedstuff (often after dehydration).

On June 12, 2003, Monsanto Philippines Inc. submitted an application to the Bureau of Plant Industry requesting for biosafety permit under AO#8 for New Leaf Plus Potato Events RBMT 21-129, RBMT 21-350 and RBMT 22-82 which has been genetically modified for Colorado Potato Beetle and Potato Leafroll Virus resistance.

Monsanto Philippines, Inc. has provided data on the identity of corn MIR 604, 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.

New Leaf Plus Potato Events RBMT 21-129, RBMT 21-350 and RBMT 22-82 has been evaluated according to BPI's safety assessment by concerned agencies: Bureau of Animal Industry (BAI) and 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 potato.

The petitioner/applicant published the Public Information Sheet (PIS) of the said application on two widely circulated newspapers (Malaya and Daily Tribune) on July 16, 2003 for public comment/review. BPI did not receive any comments 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

Potato Events RBMT 21-129, RBMT 21-350 and RBMT 22-82 (Trade name: New Leaf Plus Potato) and all potato lines/hybrids derived from these Events contain the *cry3A* coding sequence from *Bacillus thuringiensis* subsp. *tenebrionis* (*B.t.t*) strain BI256-82, which confers resistance to the Colorado potato beetle. The *Bt* gene encodes for the production of a Bt protein, which protects the plant from coleopteran insect (such as Colorado potato beetle) feeding damage. It also contains the Potato leafroll virus replicase (PLRVrep) coding sequence from the vitions of PLRV isolate LR-7, which confers resistance to the PLRV infections. The marker gene, *nptII* was inserted to aid for selection of modified plant cells. The gene products produced by the *cry 3A* and *PLRVrep* genes

provide for effective control of damage caused by CPB and PLRV respectively by inhibiting CPB feeding on potato foliage and by inhibiting the replication of PLRV in potato tissue.

Expressed Proteins

CryIII_A, nuptial, PLRV replicase for event RBMT21-129;
CryIII_A, nuptial, PLRV replicase for event RBMT 21-350; and
Cry III_A, Cp4EPSPS, PLRV replicase for event RBMT22-82.

Safety of the Expressed Proteins

The Potato Events RBMT 21-129, RBMT 21-350 and RBMT 22-82 contain the *cry 3A* coding sequence from *Bacillus thuringiensis* subsp. *tenebrionis* (*B.t.t*) that has a long history of safe use as microbial insecticide. Cry proteins have been used as safe and effective pest control agents in microbial *Bt* formulations for over forty years. The food, feed and environmental safety of the Cry3A and PLRV proteins in Potato Events RBMT 21-129, RBMT 21-350 and RBMT 22-82 was established based upon the long history of safe use of the Cry 3A protein, direct safety studies of the cry3A protein, the fact that humans and animals have safely consumed potatoes with PLRV proteins and plant virus replicase for centuries and food experts consider these infected potatoes as generally recognized as safe; lack of similarity of the two proteins to known toxins and allergens; rapid digestion of the two proteins in simulated gastric and intestinal fluids; and low dietary exposure to both Cry3A and PLRVrep proteins. The nptII protein which has no insecticidal effect, is ubiquitous in the environment and is found in microbes present on food and within the human digestive system. The *cp4 epsps* gene obtained from *Agrobacterium sp.* strain CP4 encodes the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), an enzyme of the shikimate pathway for aromatic amino acid biosynthesis in all plants, bacteria and fungi, but not animals. CP4 EPSPS protein is non allergenic. It does not share characteristics of any allergens, such as stability to digestion on acidic condition, heat stability and highly concentrated in food. The CP4 EPSPS protein shares significant amino acid sequence similar with the endogenous EPSPS present in other products such as corn and microbial and plant food products. All crop, fungal and microbial food sources such as Baker's yeast or *Bacillus subtilis* contain EPSPS proteins with history of safe use.

The *B.t.t* CPB control protein produced by New Leaf potatoes is identical to that found in nature and commercial *B.t.t* formulations.

The PLRV virions and PLRV replicase protein are routinely consumed without deleterious health effects whenever potatoes are eaten. The expected transgenic replicase protein is not detectable in New Leaf Plus Potatoes.

IV. Nutritional Composition (Compositional Analysis)

New Leaf Plus Potatoes showed that the nutritional properties of the potato tubers were substantially equivalent to those of the conventional Russet Burbank and similar white potato varieties. Key potato constituents such as total solids, sugars, vitamin C, protein, natural glykoalkaloid toxicants and proximate analysis (ash, moisture, calories) were well within the expected range for commercially produced potato tubers.

The results of compositional analysis showed that genetic improvement through the *Agrobacterium* mediated transformation of New Leaf Plus Potatoes did not alter nutrient composition from that of conventionally bred potatoes. New Leaf Plus Potatoes are as safe for consumption as conventional potatoes. There are no differences in taste or suitability for processing for New Leaf Plus Potato and the conventional potato. These imply that transformed tubers are substantially equivalent to conventional potatoes.

The risk to non-target organisms, including earthworms, beneficial insects and small mammals, were assessed to be insignificant for commercial production of NewLeaf Plus potatoes relative to standard potato varieties. The US EPA determined that the Cry3A protein is a nontoxic to non-target mammalian species based on their review of toxicity studies in mice (EPA, 2001).

V. Anti-Nutritional Factors

Anti-nutrients or toxicants called steroidal glykoalkaloids are found in most *Solanum* species. However in the New Leaf[®]Y Potatoes Events, level of glykoalcaloids is comparable to those found in non-transgenic potatoes thus no significant adverse results noted.

Potato tubers contain several protease inhibitors that inhibit the activity of trypsin, chymotrypsin and other proteases, thus decreasing the digestibility and the biological value of the ingested protein. Protease inhibitors in potatoes are largely inactivated by boiling and other thermal processes. Serious anti-nutritional reactions could occur, however, if raw or inadequately cooked potatoes are consumed or fed.

Lectins are (glyco) proteins which occur in virtually all living organisms and have the common property of binding to specific carbohydrate structures on cell surfaces, e.g. on intestinal or blood cells. Some lectins found in beans are known to cause serious health effects when ingested by humans and animals. As lectins are inactivated during heating, only consumption and feeding of raw or inadequately cooked potatoes may cause adverse effects. Negative effects of lectins on animal's health and their performance are not yet known in detail.

VI. Regulatory Decision

After reviewing the scientific data and information relevant to the application of Monsanto Philippines Inc., it is concluded that Potato Events RBMT 21-129, RBMT 21-350 and RBMT 22-82 and all progenies derived from crosses of the product with any conventionally-bred potato, and potato 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.