

Determination of the Safety of Pioneer's and Dow's
Combined Trait Product Corn: 59122 x NK 603
for Direct Use as Food or Feed or for Processing

Food and Feed Safety:

The product dossiers on Combined Trait Product Corn: 59122 x NK 603 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 combined trait product corn: 59122 x NK 603 is as safe as the conventional corn taking into account dietary impact of any changes in nutritional content or value.

A biosafety notification for combined trait product corn: 59122 x NK 603 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 or for processing was issued to Pioneer Hi-Bred Inc. and Dow Agro Sciences of the Philippines on December 15, 2006. The notification is valid for five years and shall expire on December 14, 2011 subject to the terms and conditions set forth in DA Administrative order No. 8, Series of 2002, and Memorandum Circulars Nos. 6 and 8, Series of 2004. The said combined trait product corn was included in the Lists of Approval Registry (Delisting) being 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 Combined Trait Product Corn 59122 x NK 603 in the Philippines. Food and Feed use of Combined Trait Product Corn: 59122 x NK 603 and its by-products is therefore authorized as of December 15, 2006. The biosafety notification (No. 06-012) stated that Combined Trait Product Corn 59122 x NK 603 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: Combined Trait Product Corn: 59122 x NK603

Applicant: **PIONEER HI-BRED INC. (PHI)**
Anthel Global Bldg.,
Julia Vargas St., Ortigas Center
Pasig, Metro Manila

DOW AGRO SCIENCES (DAS)
2nd Floor Bank of Commerce Building
J. Catolico Sr. Avenue, Lagao
General Santos City, South Cotabato
Telefax No. (083) 554-2290

Plant Species:

Name: Corn (*Zea mays* L.)

Parent Material: Corn 59122 and Corn NK603

Center of Origin: Mexico, Central America and 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:	Insect resistance and herbicide tolerance
Trait Introduction Method:	Conventional breeding
Donor Organism:	<p><i>Bacillus thuringiensis</i> strain PS149B1, the source of <i>cry34Ab1</i>, <i>cry35Ab1</i> which produces crystal protein effective as insecticide against specific group of insects in the <i>Diabrotica</i> genus.</p> <p><i>Streptomyces viridochromogenes</i>, the source of <i>pat</i> genes which confers tolerance to herbicidal active ingredient glufosinate-ammonium.</p> <p><i>Agrobacterium</i> sp. Strain CP4, the source of <i>cp4 epsps</i> gene which confers tolerance to glyphosate herbicides.</p>
Pathogenicity:	<p><i>B. thuringiensis</i> strain PS149B1, the source of <i>Bt</i> genes <i>cry34Ab1</i> and <i>cry35Ab1</i>—produce insecticidal proteins that are very selective in toxicity to specific organisms in the genus <i>Diabrotica</i>. Decades of safety testing on Bt proteins demonstrate the lack of toxicity to humans and animals, and the absence of adverse effects on non-target organisms and environment.</p> <p><i>Streptomyces viridochromogenes</i>, the source of <i>pat</i> gene, has no known adverse environmental or toxicological effects and has no known record toxicity, allergenicity or infectivity to human beings and animals.</p> <p><i>Agrobacterium tumefaciens</i> strain CP4, the source of CP4 EPSPS protein, is a common soil bacterium that is responsible for causing crown gall disease in susceptible plants. There have been no reports of adverse effects on humans and animals.</p>
Proposed Use:	For direct use as food, feed or for processing

II. Background Information

The approach to produce new varieties of corn stacked with insect protection and herbicide tolerance traits follows a typical breeding program. Initial crossing is conducted to introduce each transgene into a corn germplasm containing the desirable trait(s) but lacking the transgene. Once the initial cross is made, multiple generations of backcrosses to the conventional parent, followed by three or more generations of self pollination, result in progeny inbred containing the transgene in the germplasm of the parental line.

Pioneer Hi-Bred Inc. and Dow AgroSciences of the Philippines have filed an application with attached technical dossiers to the Bureau of Plant Industry on August 25, 2006 for a biosafety notification for direct use as food, feed and for processing under Administrative Order (AO) No. 8

Part 5 for combined trait product corn: 59122 x NK 603 which has been genetically modified for insect resistance and herbicide tolerance.

A safety assessment of combined trait product corn 59122 x NK 603 was conducted as per Department of Agriculture Administrative Order No. 8 Series of 2002 and Memorandum Circulars Nos. 6 and 8, Series of 2004. The focus of risk assessment is the gene interactions between the two transgenes.

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

59122 x NK 603 maize has been obtained via traditional breeding methods between progeny of two genetically modified maize lines. The two genetically modified maize lines are 59122 maize containing event DAS-59122-7, and NK603 maize containing the event MON-ØØ6Ø3-6. No new genetic modification has been introduced in 59122 x NK603

59122 maize has been genetically modified to express the Cry34Ab1, Cry35Ab1 and PAT proteins. The Cry34Ab1 and Cry35Ab1 proteins act together to control certain coleopteran pests, such as corn rootworm larvae (*Diabrotica spp.*). Expression of the PAT protein confers tolerance to the herbicidal active ingredient glufosinate-ammonium.

NK 603 maize has been genetically modified to express the protein CP4EPSPS. Expression of the CP4EPSPS protein confers tolerance to the herbicidal active ingredient glyphosate.

Two genetically modified maize lines containing events 59122 and NK 603 were crossed by conventional breeding methods to produce the combined trait product 59122 x NK 603.

Safety of Expressed Proteins

It was confirmed through Western blot analysis that no new protein was formed in 59122 x NK603 corn. The protein banding pattern and number of proteins detected in conventionally bred 59122 x NK 603 corn were the same as the parental lines (59122 x NK 603). These indicate that there is no interaction of the resulting products and no new allergen or toxin produced.

The gene products accumulate in different subcellular compartments of the plant parts. The 5-enolpyruvylshikimate-3-phosphate synthase or EPSPS is localized in plastids and encoded by nuclear genes. The enzyme catalyzes the sixth reaction of the shikimic acid pathway which is responsible for biosynthesis of aromatic compounds.

The metabolic pathways of the products are different. Because of the different sites of accumulation and mode of action of the products, unexpected effects of the stacked genes, *cry34Ab1*, *cry35Ab1*, *pat* and *cp4epsps* on the metabolism of the plant is less likely to occur. The novel proteins that are encoded by these genes do not regulate the normal metabolic pathways of plants, therefore these will not interfere with the plant's normal growth and development.

The mean expression levels of the Cry34Ab1 and Cry35Ab1 and PAT in corn grains are slightly lower in 59122 x NK603 than in 59122. PAT protein expression was very low in both corn lines. On the other hand, the expression of CP4EPSPS is slightly lower (11ng/mg) in 59122 x NK 603 than in NK603 (12.535 ng/mg). The levels, however, are within the range. Based on protein

expression analysis, the data generated from grains of 59122 x NK603 were lower than individually approved transformation events but the levels are within the range. The selectable marker, *pat* gene was transferred through conventional breeding and expressed in the plants of 59122 x NK 603. This confers tolerance to glufosinate ammonium, the active ingredient of non selective herbicide. The *cp4epsps* gene in NK603 corn which codes for CP4EPSPS that confers tolerance to glyphosate is transferred and expressed in the plants containing the stacked genes.

There is no known interaction between the *cry34Ab1*, *cry35Aab1*, *pat* and *cp4epsps* genes that gene will not be affected.

IV. Nutritional Composition (Compositional Analysis)

The World Health Organization (1995) stated that two plants that are substantially equivalent to conventional varieties are crosses by conventional breeding techniques, the combined trait product is expected to be substantially equivalent to the single event products. In accordance with OECD guideline (OECD, 2002) substantial equivalence was evaluated by comparing a) mean analyte values of the test maize to an appropriate control of similar genetic background, and b) mean proximate values of the test maize entry to analyte ranges available in the published literature.

V. Anti-Nutritional Factors

No known anti nutritional factors for individual events. Thus, 59122 x NK 603 maize has no known antinutritional factors.

VI. Regulatory Decision

After reviewing the scientific data and information relevant to the combined trait corn 59122 x NK 603 application of Pioneer Hi-Bred Inc. and Dow Agro Sciences of the Philippines, it is concluded that no interaction found between/among the combined traits, hence this plant product was found to be as safe as its conventional corn and can substitute for its traditional counterpart for direct use as food, feed and for processing and is therefore approved for direct use as food, or feed or for processing. Pioneer Hi-Bred Inc and Dow Agro Sciences are hereby notified that it may proceed with the activities for the above product for direct use as food and feed or for processing following all existing rules and regulations consistent with DA AO #8.