GM Crop Database

Database Product Description

NK603 (MON-ØØ6Ø3-6)

Host Organism *Zea mays* (Maize)

Trade Name Roundup Ready®

Trait Glyphosate herbicide tolerance.

Trait Introduction Microparticle bombardment of plant cells or tissue

Proposed Use Production for human consumption and livestock feed.

Product Developer Monsanto Company

Summary of Regulatory Approvals

Country	Food	Feed	Env	Notes
Argentina	2004	2004	2004	
Australia	2002			
Brazil	2008	2008	2008	
Canada	2001	2001	2001	
China	2005	2005		Approval renewed on 20 December 2007, valid until 20 December 2010.
Colombia	2004	2006	2007	
El Salvador	2009	2009		
European Union	2004	2004		Authorised by decision of the EU commission on July 19th, 2004 (to be effective on October 26th, 2004).
Japan	2001	2001	2004	
Korea	2002	2004		
Malaysia	2010	2010		
Mexico	2002	2002		
New Zealand	2002			
Philippines	2003	2003	2005	
Russia	2008	2004		
Singapore	2014			
South Africa	2002	2002	2002	
Taiwan	2003			
Turkey		2011		
United States	2000	2000	2000	
Uruguay	2011	2011	2011	
Vietnam	2014	2014	2014	

Introduction

Maize line NK603 was developed to allow the use of glyphosate containing herbicides as a weed control option for maize crops. The gene encoding a glyphosate tolerant form of the enzyme 5-enolpyruvlyshikimate-3-phosphate synthase (EPSPS) was isolated from the soil bacterium *Agrobacterium tumefaciens* strain CP4 and introduced into maize using recombinant DNA techniques. Glyphosate specifically binds to and inactivates EPSPS, which is involved in the biosynthesis of the aromatic amino acids tyrosine, phenylalanine and tryptophan. This enzyme is present in all plants, bacteria and fungi, but not in animals, which do

not synthesize their own aromatic amino acids. Thus, EPSPS is normally present in food derived from plant and microbial sources.

For environmental release in the United States, another glyphosate tolerant maize line, GA21, was designated as the antecedent organism for NK603. Maize line NK603 and the antecedent organism GA21 were genetically engineered using the same transformation method and contain a functionally equivalent enzyme that makes the plants tolerant to the herbicide glyphosate.

Summary of Introduced Genetic Elements

Code	Name	Type	Promoter, other	Terminator	Copies	Form
CP4 epsps	5-enolpyruvyl shikimate-3-phosphate synthase	HT	P-ract1/ract1 intron containing rice actin 1 promoter, transcription start site	A. tumefaciens nopaline synthase (nos) 3'-untranslated region	1	CP4 EPSPS gene modified for plant-preferred codons
CP4 epsps	5-enolpyruvyl shikimate-3-phosphate synthase	HT	enhanced CaMV 35S, maize HSP70 intron	A. tumefaciens nopaline synthase (nos) 3'-untranslated region	1	CP4 EPSPS gene modified for plant-preferred codons

Characteristics of *Zea mays* L. (Maize)

Center of Origin	Reproduction	Toxins	Allergenicity
Mesoamerican region, now Mexico and Central America	Cross-pollination via wind-borne pollen is limited, pollen viability is about 30 minutes. Hybridization reported with teosinte species and rarely with members of the genus *Tripsacum*.	No endogenous toxins or significant levels of antinutritional factors.	Although some reported cases of maize allergy, protein(s) responsible have not been identified.

Donor Organism Characteristics

Latin Name	Gene	Pathogenicity
Agrobacterium tumefaciens strain CP4	CP4 epsps	*Agrobacterium tumefaciens* 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 or animals.

Modification Method

Nutritional Data

Compositional analyses were performed on grain and forage samples of NK603 (treated with glyphosate) and the non-transformed parental control line together with a number of other commercial maize hybrids planted at trial sites in the U.S. and Europe. Analyses of grain samples included measurements of proximates (protein, fat, ash, moisture), acid detergent fibre (ADF), neutral detergent fibre (NDF), amino acids, fatty acids, vitamin E, minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc), and the antinutritional

components, phytic acid and trypsin inhibitor.

In all of these tests, small statistical differences between NK603 and control lines were observed only in: six amino acids (alanine, arginine, glutamic acid, histidine, lysine, and methionine) as measured in grain from European trials (no differences were observed in material from U.S. trials); and stearic (C18:0) acid levels. Overall, these differences were not consistent across all trial sites and they were considered to reflect random variation. All compositional results were within the ranges observed for commercial non-transformed lines.

The nutritional quality of NK603 grain was assessed in feeding trials with broiler chickens, finisher swine, and laboratory rats. These studies showed that there were no differences between the transformed and non-transformed maize.

Toxicity

The CP4 EPSPS gene encodes a single polypeptide of 455 amino acids (47.6 kDa) which exhibits about 50% amino acid sequence similarity with the analogous plant EPSPS enzyme. The family of bacterial and plant EPSPS proteins are not known to display any toxic or allergenic properties. The potential toxicity of the CP4 EPSPS protein was assessed by comparing its amino acid sequence against a database of 4,677 protein sequences (not all unique) that have been associated with toxicity, and in an acute oral toxicity study in mice. The CP4 EPSPS protein did not display any sequence homology with known protein toxins and did not result in any adverse effects on test animals (50 males, 50 females) receiving doses up to 400 mg/kg of bacterially derived CP4 EPSPS protein. The single amino acid substitution within the CP4 EPSPS L214P protein did not alter the sequence comparison results.

Allergenicity

The CP4 EPSPS encoding gene was not derived from an organism known to cause allergic reactions and the allergenic potential of this protein was further evaluated by comparing its amino acid sequence against a database of known allergens, and by assessing its stability to digestion in the presence of simulated gastric fluids. There was no sequence homology between CP4 EPSPS and known allergens when checked against a database of 567 protein sequences using an 8-amino acid length window. As assessed by Western immunoblot analysis, the CP4 EPSPS was rapidly degraded (T50 < 15 sec) upon exposure to pepsin-containing simulated gastric fluid or trypsin-containing simulated intestinal fluid (T50 <= 10 min). Similar results were obtained with the variant CP4 EPSPS L214P protein.

Characteristics of the Modification

The Introduced DNA
The incorporated DNA was characterized using a combination of Southern blot analyses, polymerase chain reaction (PCR) amplification of specific sequences, and nucleotide sequencing of the entire inserted fragment, including flanking sequences from the host genome. In addition, bioinformatics analyses were conducted on the inserted sequence, including host genome junction regions, to demonstrate the lack of any unforeseen, or chimeric, open reading frames (ORFs) that could potentially result in the expression of unanticipated novel proteins.

These analyses demonstrated the introduction of single copy of the transforming DNA at a single insertion site within the host genome. Some anomalies were, however, observed:

- In addition to a complete copy of the introduced DNA, the insert also included a 217 bp fragment (50 bp of polylinker sequence + 167 bp of the enhancer region of the rice actin promoter) inversely linked to the 3? terminus of the introduced DNA.
- Nucleotide sequencing indicated that the sequence of the CP4 EPSPS gene within the second (3? proximal) cassette differed by two nucleotides from the inserted sequence. This gave rise to a single amino acid substitution at position 214 of the expressed protein (leucine --> proline; variant protein referred to as: CP4 EPSPS L214P).
- Analyses of specific PCR products from the 3? terminus of the inserted DNA revealed that an additional segment comprising 305 bp of chloroplast DNA had been co-integrated. Bioinformatics analysis indicated that this sequence corresponded to a portion of the maize DNA-directed RNA polymerase alpha-subunit and ribosomal S11 protein. The source of this DNA was believed to be the chloroplast of the transformed cell.

Genetic Stability of the Introduced Trait
Southern blot analyses of genomic DNA isolated from plants over six generations of crossing and three generations of self-pollination were used to confirm that the introduced DNA was stably inherited and segregated as a single locus according to Mendelian genetics. Multigenerational stable expression of the glyphosate tolerant trait was demonstrated using bioassay (tolerance to glyphosate application) and enzyme linked immunosorbent assay (ELISA) to measure CP4 EPSPS protein concentration.

Expressed Material
Expression of full-length (approx. 47 kDa) CP4 EPSPS protein was confirmed by Western immunoblot analysis and protein concentrations were estimated using ELISA. Plant samples from line NK603 and the non-transformed parental control line were collected from six non-replicated and two replicated field sites during the 1998 growing season and assayed using a double antibody sandwich (DAS)-ELISA employing monoclonal anti-CP4 EPSPS antibody as the capture antibody and a horseradish peroxidase-conjugated anti-CP4 EPSPS polyclonal antibody as the detection reagent. Mean CP4 EPSPS protein levels (across all sites) were 25.6 ?/g (fwt; range 18.0 ? 31.2) and 10.9 ?/g (fwt; range 6.9 ? 15.6) for forage and grain tissue, respectively.

Environmental Safety Considerations

Outcrossing
Pollen production and viability were unchanged in line NK603 and, therefore, pollen dispersal by wind and outcrossing frequency should be no different than for other maize varieties. Gene exchange between NK603 and other cultivated maize varieties will be similar to that which occurs naturally between cultivated maize varieties at the present time. In Canada and the United States, where there are few plant species closely-related to maize in the wild, the risk of gene flow to other species is remote. Cultivated maize, *Zea mays* L. subsp. mays, is sexually compatible with other members of the genus Zea, and to a much lesser degree with members of the genus Tripsacum. None of the sexually compatible relatives of maize in Canada or the United States are considered to be weeds in these countries and it is therefore unlikely

that introgression of the CP4 EPSPS gene would provide a selective advantage to these populations as they would not be routinely subject to herbicide treatments.

Weediness Potential
No competitive advantage was conferred to NK603, other than that conferred by resistance to glyphosate herbicide. Resistance to glyphosate containing herbicides will not, in itself, render maize weedy or invasive of natural habitats since none of the reproductive or growth characteristics were modified.
Cultivated maize is unlikely to establish in non-cropped habitats and there have been no reports of maize surviving as a weed. In agriculture, maize volunteers are not uncommon but are easily controlled by mechanical means or by using herbicides that are not based on glyphosate as appropriate. *Zea mays* is not invasive and is a weak competitor with very limited seed dispersal.

Secondary and Non-Target Adverse Effects
No environmentally toxic components were detected in NK603. CP4 EPSPS protein is not a known toxin and analogous proteins are found in all plants and microorganisms. There are no anticipated adverse effects of NK603 on non-target organisms that would be different from conventional maize varieties.

Impact on Biodiversity
Maize line NK603 has no novel phenotypic characteristics that would extend its use beyond the current geographic range of maize production. Since the risk of outcrossing with wild relatives in Canada and the United States is remote, it was determined that the risk of transferring genetic traits from NK603 to species in unmanaged environments was not a significant concern.

Other Considerations
Consideration was made as to whether the introduction of crops tolerant to glyphosate would result in a significant increase in the use of the herbicide, and lead to the evolution of glyphosate resistant weeds. It was determined that the risk of increasing the selection of glyphosate tolerant weeds was low and could be mitigated through the use of other approved herbicides with a mode of action dissimilar to glyphosate.

Food and/or Feed Safety Considerations

Nutritional Data
Compositional analyses were performed on grain and forage samples of NK603 (treated with glyphosate) and the non-transformed parental control line together with a number of other commercial maize hybrids planted at trial sites in the U.S. and Europe. Analyses of grain samples included measurements of proximates (protein, fat, ash, moisture), acid detergent fibre (ADF), neutral detergent fibre (NDF), amino acids, fatty acids, vitamin E, minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc), and the antinutritional components, phytic acid and trypsin inhibitor.

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Abstract

Corn line NK603 was developed to allow the use of the herbicide glyphosate as a weed control option in corn. The gene conferring tolerance to glyphosate was introduced via genetic engineering techniques. These techniques enable the developer to express in the corn plant the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) gene, which is involved in the synthesis of aromatic amino acids. This gene, isolated from Agrobacterium tumefaciens strain CP4 (CP4 EPSPS), encodes a version of the enzyme that is tolerant to concentrations of glyphosate that would normally inhibit the activity of the endogenous corn enzyme. The CP4 EPSPS gene and regulatory sequences controlling its expression were introduced via biolistic particle bombardment.

Line NK603 was produced from a proprietary inbred line designated as AW x CW using particle acceleration. NK603 contains two plant expression cassettes each containing a single copy of the CP4 EPSPS gene and respective regulatory sequences as follows:

CP4 EPSPS Cassette 1 contains the CP4 EPSPS gene under the control of the rice actin 1 promoter (McElroy et al. 1990) and the CTP2 chloroplast transit peptide leader sequence from Arabidopsis thaliana (Klee and Rogers 1987). The purpose of this latter sequence is to direct CP4 EPSPS protein to the chloroplast, which is the site of aromatic amino acid synthesis. The CP4 EPSPS gene sequence was modified slightly, but retains greater than 99.4% homology to the native Agrobacterium gene. The NOS 3' nontranslated region of the nopaline synthase gene from Agrobacterium tumefaciens T-DNA was used to provide the polyadenylation signal.

CP4 EPSPS Cassette 2 contains the CP4 EPSPS gene under the control of the enhanced cauliflower mosaic virus (CaMV) 35S promoter (Kay et al. 1985). The Zmhsp70 intron from the corn hsp70 heat shock protein was included to stabilize the level of transcription (Rochester et al. 1986) and the CTP2 chloroplast transit peptide leader sequence was used to direct the CP4 EPSPS protein to the chloroplast. The NOS 3' nontranslated region of the nopaline synthase gene from Agrobacterium tumefaciens T-DNA was used to provide the polyadenylation signal.

Roundup ready corn line GA21 was designated as the antecedent organism for NK603, which was not judged to have any characteristics that would pose a greater impact on the environment or non-target organisms.

References:

Kay, R., Chan, A., Daly, M. & McPherson, J. (1985). Duplication of the CaMV 35S promoter sequences creates a strong enhancer for plant genes. Science 236: 1299-1302.

Klee, H.J. & Rogers, S.G. (1987). Cloning of an Arabidopsis gene encoding 5-enolpyruvlyshikimate-3-phosphate synthase: sequence analysis and manipulation to obtain glyphosate-tolerant plants. Mol. Gen. Genet. 210: 437-442.

McElroy, D., Zhang, W., Cao, J. & Wu, R. (1990). Isolation of an efficient actin promoter for use in rice transformation. Plant Cell 2: 163-171.

Links to Further Information

Australia New Zealand Food Authority

Final risk assessment report: glyphosate-tolerant corn line NK603 (http://www.cera-gmc.org/files/cera/GmCropDatabase/docs/decdocs/02135005.pdf) [PDF Size: 425.38K bytes]

Canadian Food Inspection Agency, Plant Biosafety Office

Decision Document DD2002-35 Determination of the Safety of Monsanto Canada Inc.’s Roundup ReadyTM Corn (Zea mays L.) Line 603 (http://www.cera-gmc.org/files/cera/GmCropDatabase/docs/decdocs/02-022-001.pdf) [PDF Size: 37.13K bytes]

Comissão Técnica Nacional de Biossegurança - CTNBio (Brazil)

Risk Assessment of Herbicide Tolerant Maize (NK603) (http://www.cera-gmc.org/files/cera/GmCropDatabase/docs/decdocs/09-060-005.pdf) [PDF Size: 487.58K bytes]

European Commission

COMMISSION DECISION of 3 March 2005 authorising the placing on the market of foods and food ingredients derived from genetically modified maize line NK 603 as novel foods or novel food ingredients under Regulation (EC) No 258/97 of the European Parliament and of the Council (http://www.cera-gmc.org/files/cera/GmCropDatabase/docs/decdocs/06-286-015.pdf) [PDF Size: 44.27K bytes]

European Commission: Community Register of GM Food and Feed

Notification of the placing on the Community Register of MON-ØØ6Ø3-6. (http://www.cera-gmc.org/files/cera/GmCropDatabase/docs/decdocs/06-286-017.pdf) [PDF Size: 11.49K bytes]

European Food Safety Authority

Opinion of the Scientific Panel on Genetically Modified Organisms on a request from the Commission related to the safety of foods and

food ingredients derived from herbicide-tolerant genetically modified maize NK603, for which a request for placing on the market was submitted under Article 4 of the Novel Food Regulation (EC) No 258/97 by Monsanto (QUESTION NO EFSA-Q-2003-002). Opinion adopted on 25 November 2003 (http://www.cera-gmc.org/files/cera/GmCropDatabase/docs/decdocs/06-286-016.pdf) [PDF Size: 181.57K bytes]

Health Canada, Office of Food Biotechnology

Novel food decision summary for glyphosate-tolerant maize line NK603 (http://www.cera-gmc.org/files/cera/GmCropDatabase/docs/decdocs/nk603-hcds.pdf) [PDF Size: 15.13K bytes]

Japanese Biosafety Clearing House, Ministry of Environment

Outline of the biological diversity risk assessment report: Type 1 use approval for NK603 (http://www.cera-gmc.org/files/cera/GmCropDatabase/docs/decdocs/06-291-003.pdf) [PDF Size: 130.40K bytes]

Monsanto Company

Product safety description (http://www.cera-gmc.org/files/cera/GmCropDatabase/docs/decdocs/02-269-007.pdf) [PDF Size: 275.70K bytes]

Philippines Department of Agriculture, Bureau of Plant Industry

Determination of the Safety of Monsanto’s Corn NK 603 (Glyphosate-Tolerant Corn) for Direct Use as Food, Feed and for Processing and for Propagation (http://www.cera-gmc.org/files/cera/GmCropDatabase/docs/decdocs/09-131-005.pdf) [PDF Size: 30.90K bytes]

U.S.Department of Agriculture, Animal and Plant Health Inspection Service

Monsanto Co. Request for Extension of Determination of Nonregulated Status to the Additional Regulated Article: Roundup Ready Corn Line NK603 (http://www.cera-gmc.org/files/cera/GmCropDatabase/docs/decdocs/05-242-041.pdf) [PDF Size: 4.47M bytes]

US Food and Drug Administration

Letter to Monsanto regarding NK603 (http://www.cera-gmc.org/files/cera/GmCropDatabase/docs/decdocs/bnfL071.pdf) [PDF Size: 84.06K bytes]
Memorandum to file concerning glyphosate-tolerant maize link NK603. (http://www.cera-gmc.org/files/cera/GmCropDatabase/docs/decdocs/bnfM071.pdf) [PDF Size: 376.82K bytes]