FOOD DERIVED FROM INSECT-PROTECTED AND GLUFOSINATE AMMONIUM-TOLERANT DBT418 CORN

A Safety Assessment

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SUMMARY AND CONCLUSIONS

Food from insect-protected and glufosinate ammonium-tolerant DBT418 corn has been evaluated to determine its safety for human consumption. The evaluation criteria used in this assessment included: a characterisation of the genes, their origin and function; the changes at the DNA, protein and whole food levels; stability of the introduced genes in the corn genome; compositional analyses; evaluation of intended and any unintended changes; and the potential of the newly expressed proteins to be allergenic or toxic.

History of use

Corn (*Zea mays* L.) is used as a staple food by a significant proportion of the world's population. Corn-based products are routinely used in a large number and diverse range of foods, and have a long history of safe use. Products derived from DBT418 corn may include highly processed corn products such as flour, breakfast cereals, high fructose corn syrup and other starch products.

Nature of the genetic modification

Insect-protected and glufosinate ammonium-tolerant DBT418 corn was generated through the transfer of the *cryIAc* and *bar* genes to the inbred corn line, AT824. The *cryIAc* gene is derived from *Bacillus thuringiensis* subspecies *kurstaki* and encodes the insecticidal crystal protein CryIAc, the toxic effect of which is specific to Lepidopteran insects, including the European corn borer (ECB). The *bar* gene is derived from *Streptomyces hygroscopicus* and encodes the enzyme phosphinothricin acetyltransferase (PAT) which inactivates phosphinothricin (PPT), the active constituent of glufosinate ammonium herbicides. The herbicide tolerant trait was used as a marker to facilitate the selection of transformed cells from non-transformed cells during the plant transformation procedure and is not exploited commercially in DBT418 corn.

Other genes transferred along with the *cryIAc* and *bar* genes were *bla* and *pinII*. The *bla* gene is derived from *Escherichia coli* and is used as a marker to select transformed bacteria from non-transformed bacteria during the DNA cloning and recombination steps undertaken in the laboratory prior to transformation of the plant cells. It codes for the enzyme β-lactamase and confers resistance to a number of β-lactam antibiotics such as ampicillin. The *pinII* gene is derived from potato (*Solanum tuberosum* L.) and encodes a serine protease inhibitor that is reported to enhance the insecticidal activity of CryIAc against various lepidopteran pests. The *pinII* gene in DBT418 corn is non-functional and does not give rise to any protein products.

Molecular and genetic analyses of the DBT418 corn indicate that the transferred genes are stably integrated into the plant genome and are stably inherited from one generation to the next.

One of the important issues to consider in relation to genetically modified foods is the impact on human health from potential transfer of antibiotic resistance genes to microorganisms in the human digestive tract. In the case of DBT418 corn, it was concluded that the *bla* gene would be extremely unlikely to transfer to bacteria in the

human digestive tract because of the number and complexity of the steps that would need to take place consecutively. More importantly however, in the highly unlikely event that transfer did occur, the human health impacts would be negligible because ampicillin resistant bacteria are already commonly found in the human gut and in the environment.

Characterisation of novel protein

DBT418 corn was shown to produce two new proteins at very low levels – CryIAc and PAT. PAT is expressed at significantly higher levels than CryIAc in DBT418 corn. In kernels, mean CryIAc levels ranged from 36.0 – 42.8ng/g dry weight (equivalent to about 0.0001% of the total protein) and mean PAT levels ranged from 3.1 – 6.0µg/g dry weight (equivalent to about 0.0175% of the total protein). Higher levels of CryIAc and PAT were detected in other parts of the plant, particularly the leaves, however these are not used for human consumption.

The newly expressed CryIAc and PAT proteins in DBT418 corn were evaluated for their potential to be toxic to humans using acute toxicity testing in animals. For CryIAc, no deaths or other adverse signs were recorded in mice at doses up to 3825mg/kg bodyweight. In a similar study using PAT, no deaths or other adverse signs were recorded at doses up to 2500mg/kg bodyweight. No deaths or other adverse signs were also observed in an acute toxicity study with birds using 200 000ppm of lyophilised DBT418 leaf tissue. As the CryIAc and PAT expression levels in corn kernels are low, exposure to both proteins through the consumption of DBT418 corn products would be very low, and certainly well below the levels found to be safe in acute toxicity tests using animals.

The potential allergenicity of the novel proteins was investigated by evaluating whether either of the proteins exhibited any of the characteristics of known allergens. Both proteins are rapidly digested in simulated mammalian digestive systems and a comparison of their amino acid sequence with that of known allergens did not reveal any biologically or immunologically significant similarities. Furthermore, both proteins are expressed in corn kernels at low levels indicating there would be little potential for allergic sensitisation.

The evidence does not indicate that there is any potential for either CryIAc or PAT to be toxic to humans and also indicates that both proteins have limited potential as food allergens.

Comparative analysis

Compositional analyses were done to establish the nutritional adequacy of DBT418 corn, and to compare it to non-transformed control lines. The components measured were protein, oil, moisture, starch, fibre, ash, fatty acids, amino acids, as well as the minerals phosphorous and calcium. No significant differences in the levels of these major constituents or nutrients between transgenic and control lines were observed. Therefore, on the basis of the data submitted in the present application, DBT418 corn can be considered compositionally no different to other commercial corn varieties.

Conclusion

No potential public health and safety concerns have been identified in the assessment of DBT418 corn. Therefore, on the basis of the data provided in the present application, and other available information, foods derived from DBT418 corn can be considered as safe and wholesome as foods derived from other corn varieties.

INTRODUCTION

A safety assessment has been conducted on food derived from corn, genetically modified to be protected from lepidopteran insects, particularly the European corn borer, and tolerant to the herbicide glufosinate ammonium. The corn is commonly known as 'DBT418 corn' and when DBT418 hybrids are sold commercially the suffix 'BtX' is incorporated into the name of the hybrid corn (e.g. DK493BtX).

Protection against European corn borer (*Ostrinia nubilalis*) is achieved through expression in the plant of a protein – called CryIAc – that is produced naturally by the *kurstaki* subspecies of the spore-forming soil bacterium *Bacillus thuringiensis*. The majority of described *B. thuringiensis* strains produce proteins that have insecticidal activity against lepidopteran insects (larvae of moths and butterflies) although a few have activity against dipteran (mosquitos and flies) and coleopteran (beetles) insects. Microbial pesticide products based on *B. thuringiensis* producing CryIAc (e.g. DIPEL®) have been approved for use on a variety of crops and for home garden use and have been available in both Australia and New Zealand since 1989.

Tolerance to glufosinate ammonium is achieved through expression in the plant of the enzyme phosphinothricin acetyl transferase (PAT). PAT inactivates phosphinothricin (PPT), the active constituent of glufosinate ammonium. Glufosinate-ammonium is currently registered in Australia under the commercial name of Basta® for non-selective uses, or Finale® for turf and home garden uses, and as Buster® in New Zealand. The herbicide tolerant trait of DBT418 corn is not exploited commercially and was incorporated into the corn for selection purposes only.

Corn varieties containing the DBT418 transformation event were developed for cultivation in the United States. This variety has since been discontinued, its last planting being in 1999, however as significant quantities were planted in its final year of production, there is still potential for DBT418 corn to be present in corn products imported into Australia and New Zealand from the United States. The major imported corn product is high-fructose corn syrup, which is not currently manufactured in either Australia or New Zealand. Corn products are processed into breakfast cereals, baking products, extruded confectionary and corn chips. Other corn products, including maize starch used by the food industry for the manufacture of dessert mixes and canned food, are also imported.

HISTORY OF USE

Corn (*Zea mays* L., also called maize) has been cultivated for centuries and is used as a basic food item by people throughout the world. A large part of corn production is used for human food products, and a wide variety of food products are derived from corn kernels. Grain and by-products from processing of corn are also used as animal feedstuffs.

In developed countries, corn is consumed mainly as popcorn, sweet corn, corn snack foods and occasionally as corn bread. However, most consumers are not aware that

corn is an important source of the sweeteners, starches, oil and alcohol used in many foods, beverages and numerous other products.

Two milling procedures are used for the processing of corn: dry milling and wet milling. Dry milling is a mechanical process in which the endosperm is separated from the other components of the kernels and fractionated into coarse particles (grits). The process is used to produce meal and flour for use in cereals, snack foods and bakery products, or for use in brewing (Alexander 1987). Human food products derived from dry milling include corn flakes, corn flour and grits.

The wet milling process for corn is designed to physically separate the major component parts of the kernel: starch, protein, oil and fibre. Wet milling produces primarily starch (typically 99.5% pure). In this process grain is steeped in slightly acidic water for 24–48 hours at 52°C before being milled. Starch is separated from other solids through a number of grinding, washing and sieving steps. Washed starch may contain 0.3-0.35% total protein and 0.01% soluble protein (May 1987). Starch is largely converted to a variety of products for human consumption, such as sweetener and fermentation products including high fructose corn syrup and ethanol. Oil is produced from wet-milled corn by solvent extraction and heat (120°C, May 1987) and corn oil is considered to be free of protein.

In Australia and New Zealand crop planting regimes are variable. Due to the diverse uses of corn products, there is a requirement to import corn products, mainly in the form of high-fructose corn syrup, to meet manufacturing demand.

DESCRIPTION OF THE GENETIC MODIFICATION

Methods used in the genetic modification

DBT418 corn was produced by the simultaneous introduction of DNA from three different plasmids (pDPG699, pDPG165 and pDPG320) into embryogenic cells of the inbred corn line AT824 using the technique of microprojectile bombardment (Gordon-Kamm *et al* 1990).

Function and regulation of the novel genes

Transformation of corn with plasmids pDPG699, pDPG165 and pDPG320 resulted in the transfer of three gene expression cassettes — *cryIAc*, *bar* and *pinII*. Each of these expression cassettes is described in Table 1.

Table 1: Gene expression cassettes in pDPG699, pDPG165 and pDPG320

Cassette	Genetic element	Source	Function
pDPG699:			
CryIAc	OCS-35S promoter	OCS is a 20 bp enhancer sequence derived from the T-DNA of *Agrobacterium tumefaciens* (Benfey and Chua 1990, Bouchez *et al* 1989). Two copies of OCS were positioned upstream of the 90 bp A domain of the cauliflower mosaic virus (CaMV) 35S promoter (Odell *et al* 1985).	A chimeric promoter for high level gene expression in plant cells. The OCS enhancer is known to promote expression of genes in most vegetative plant tissues.
	adh1 intron VI	The intron VI from the maize alcohol dehydrogenase I (*adh1*) gene (Dennis *et al* 1984).	Used to improve transcription of the *cryIAc* gene.
	cryIAc	Synthetic gene encoding the first 613 amino acids of the HD73 CryIAc endotoxin from *B. thuringiensis* (Adang *et al* 1985).	Confers protection against lepidopteran insects, including the European corn borer.
	pinII 3'	The putative 3' untranslated region and transcription termination region of the protease inhibitor II (*pinII*) gene from potato (Thornburg *et al* 1987).	Contains signals for termination of transcription and directs polyadenylation.
pDPG165:			
bar	35S promoter	A promoter derived from the cauliflower mosaic virus (Odell *et al* 1985).	A promoter for high-level constitutive gene expression in plant tissues.
	bar	Gene from *Streptomyces hygroscopicus* encoding phosphinothricin acetyltransferase (De Block *et al* 1987, White *et al* 1990).	Confers tolerance to phosphinothricin, the active constituent of glufosinate ammonium herbicides.
	Tr7 3'	The 3' untranslated region from *A. tumefaciens* T-DNA transcript 7 (Dhaese *et al* 1983).	Contains signals for termination of transcription and directs polyadenylation.
pDPG320:			
pinII	35S promoter	As above.	As above.
	adhI intron I	The first intron from the maize *adhI* gene (Dennis *et al* 1984).	As above.
	pinII	Gene from potato encoding protease inhibitor II (Thornburg *et al* 1987).	Inhibits serine proteases and has been shown to inhibit both trypsin and chymotrypsin (Ryan 1990).
	Tr7 3'	As above.	As above.

The cryIAc gene

The *cryIAc* gene used is a synthetic version of the native c*ry1Ac* gene derived from the soil bacterium *B. thuringiensis* subsp. *kurstaki* strain HD73 (Adang *et al* 1985). The gene is one of several that have been isolated from *B. thuringiensis* species, which encode a group of proteins known as the δ-endotoxins or the crystal proteins. Most crystal proteins are synthesised intracellularly as inactive protoxins that spontaneously form small crystals, approximately 1µm in size. These proteins are selectively active against several Orders of insects such as the Lepidoptera, Coleoptera, and Diptera. The crystal proteins are produced by the bacterium during sporulation. The protein product of the *cryIAc* gene, CryIAc, is selectively active against Lepidopteran insects (MacIntosh *et al* 1990b).

When ingested by susceptible insect species, the highly alkaline pH of the insect midgut promotes solubilisation of the protoxin–containing crystals. The protoxin is then activated by trypsin–like proteases in the insect gut which cleave off domains from the carboxy and amino–termini leaving a protease–resistant core representing the active protein. The active protein binds to highly specific glycoprotein receptors on the surface of the midgut epithelial cells in the insect (Rajamohan 1998). This binding of the protein to specialised receptors has been shown to be essential for the onset of toxicity (Wolfersberger 1990, Ferré *et al* 1991). Aggregation of the protein molecules results in formation of a pore through the cell membrane. These cells eventually swell and burst, causing loss of gut integrity and resulting in larval death within 1 to 2 days (Hofte and Whitely 1989, Schnepf *et al* 1998).

The bacterial c*ry1Ac* gene has a high content of the nucleotides guanosine (G) and cytosine (C) that is not typical of plant genes, so it is not well expressed in plants. To optimise its expression in plant cells the native *cryIAc* gene was re-synthesised to lower the GC content. This was achieved without altering the amino acid sequence so the synthetic gene encodes a protein that is identical to the first 613 amino acids of the native bacterial CryIAc protein.

The bar gene

The *bar* gene, encoding phosphinothricin acetyl transferase (PAT), has been cloned from the soil bacterium *Streptomyces hygroscopicus* (ATCC 21705) (De Block *et al* 1987) and its full DNA sequence of 549 base pairs has been published (White *et al* 1990). The GTG translation initiation codon present in the native *bar* gene from *S. hygroscopicus* was mutated to ATG to conform to plant codon usage.

PAT is produced by *S. hygroscopicus* to protect itself from the toxicity of the antibiotic (phosphinothricin alanyl alanine or bialaphos) that it produces. The PAT enzyme catalyses two reactions in the bacterium: the acetylation of demethylphosphinothricin, which is an intermediate step in the biosynthesis of bialaphos; and the acetylation of phosphinothricin, which is the activity that serves to protect *S. hygroscopicus* from phosphinothricin toxicity.

Phosphinothricin (PPT), the active ingredient of glufosinate ammonium, was initially characterised as bialaphos produced by another bacterium *Streptomyces viridochromogenes* (Comai and Stalker 1986) and was later shown to be effective as a broad-spectrum herbicide. PPT can also be chemically synthesised. PPT is a potent competitive inhibitor of glutamine synthase (GS; EC 6.3.1.2) in plants. GS plays a central role in the assimilation of ammonia and in the regulation of nitrogen metabolism in plants.

It is the only enzyme in plants that can detoxify ammonia released by nitrate reduction, amino acid degradation and photorespiration. Inhibition of GS in plants by PPT causes rapid accumulation of ammonia leading to cell death (De Block *et al* 1987).

In DBT418 corn, the *bar* gene acts only as a selection marker, allowing plants to be distinguished from non-transformed plants; DBT418 corn is not marketed as a herbicide-tolerant variety.

The pinII gene

The *pinII* gene, encoding an inhibitor of serine proteases, was originally cloned from potato (Thornburg *et al* 1987). The encoded protein, referred to as protease inhibitor II, contains two active sites, one of which inhibits trypsin and the other which inhibits chymotrypsin (Plunkett *et al* 1982). In potato, the protease inhibitor protein is naturally expressed in leaves in response to chewing insects or other severe mechanical damage, and is thought to help defend the plant against insect predators by reducing the digestibility and nutritional quality of the leaves (Ryan 1978).

The *pinII* gene was transferred into DBT418 corn because it had been reported that high level expression of protease inhibitor II in tobacco plants had conferred resistance to a lepidopteran pest, *Manduca sexta* (Johnson *et al* 1989) and that the presence of serine protease inhibitors has served to enhance the insecticidal activity of the crystal proteins from *B. thuringiensis* subsp. *kurstaki* (MacIntosh *et al* 1990a).

Other genetic elements

The plasmid vectors also each contained a number of additional genetic elements and these are described in Table 2 below. These genetic elements are present in most *Escherichia coli* cloning vectors and are well described (Sambrook *et al* 1981). They are used to assist in the manipulation of DNA sequences as well as direct gene expression in *E. coli*.

Table 2: Additional genetic elements in plasmids pDPG699, pDPG165 and pDPG320

Genetic element	Source	Function
lac	An incomplete copy of the *lac* operon which contains a partial *lac* repressor (*lac*I) coding sequence, the promoter P_{lac}, and a partial coding sequence for β-galactosidase (*lac*Z) from the phagemid pBluescripte SK(-) (Stratagene).	Sequences used to assist in the cloning of genes into plasmids.
fl (-) ori (not in pDPG165)	Bacteriophage f1 origin of replication from phagemid pBluescript SK(-) (Stratagene).	Used to produce single stranded DNA. The f1 origin is not recognised unless bacteriophage f1 is present.
bla	The β-lactamase gene from phagemid pBluescript SK(-) (Stratagene).	Confers resistance to ampicillin and other penicillins (Sutcliffe 1978).
ColE1 ori	Plasmid origin of replication from the *Escherichia coli* high copy phagemid pBluescript SK(-) (Stratagene).	Allows plasmids to replicate in *E. coli.*

The *bla* gene is derived from the bacterium *Escherichia coli* and encodes the enzyme β-lactamase that confers resistance to a number of β-lactam antibiotics, including the moderate-spectrum penicillin, and ampicillin. The *bla* gene is under the control of a bacterial promoter and was included as a marker to allow for selection of bacteria containing pDPG699, pDPG165 and pDPG320 prior to transformation of the plant cells. Bacterial cells are plated onto medium containing ampicillin, and only those that have been transformed with the plasmid conferring antibiotic resistance will grow. As the *bla* gene is under the control of a bacterial promoter it is therefore not expressed in transformed plant cells.

Characterisation of the genes in the plant

Studies evaluated:

Stephens, M. *et al* (1996). Molecular characterization of transgene content and stability in transgenic corn hybrid line DK.DL (DBT418). Performing laboratory: DEKALB Genetics Corporation. Study No. DGC-95-A07.

Albee, L.D. *et al* (2001). Amended report for: Confirmation of the genomic DNA sequences flanking the 5' and 3' ends of the insert in corn event DBT418. Performing laboratory: Monsanto Company. Study No. 00-01-39-52.

Selection and derivation of plant lines

A transformed callus line, designated DBT418, was selected and individual plants were regenerated. Regenerated DBT418 plants (referred to as the T_0 generation) were then crossed with non-transformed, inbred corn lines to produce T_1 seed inheriting the DBT418 transformation event. Repeated backcrossing to various inbred lines resulted

in hybrid germplasm containing the DBT418 transformation event. During the backcross program, segregating populations of plants were sprayed with glufosinate ammonium to identify positive segregants. Typically, about one half of the plants resulting from a backcross were found to be tolerant to the herbicide. The herbicide tolerant plants were also protected from European corn borer infestations, indicating that both genes were linked, possibly resulting from the same insertion event.

Characterisation of DBT418 corn

The DBT418 transformation event was created by the introduction of the three plasmids — pDPG165, pDPG320 and pDPG699. These plasmids encode the *bar*, potato *pinII*, and *cryIAc* genes, respectively. In addition, each plasmid contains the *bla* gene as well as the ColE1 origin of replication. The DBT418 transformation event was characterised using Southern blot, polymerase chain reaction (PCR) and nucleotide sequence analyses.

The maize genotype used as the test substance for this study is designated DK.DL(DBT418) and is the result of a cross between a female inbred line, DK, that is homozygous for the DBT418 event, and a male inbred line, DL, that does not contain the DBT418 event. Consequently, the resultant test plant, DK.DL(DBT418) is hemizygous for the DBT418 event. The control used for this study was non-transformed hybrid seed of the genotype DK.DL. In both the Southern and PCR analyses, plasmids bearing the target sequences were used as positive control references. Leaf material for genomic DNA extraction was harvested from each germinated plant between 54 and 60 days post-planting.

The Southern blot analyses were primarily used to determine the copy number of each transferred gene. Copy number was determined by comparing the hybridisation signal for each genetic element with standards prepared from each of the plasmids diluted to represent 0.5, 1, 2, 4 and 8 maize genome copy number equivalents.

The results of the Southern blot analyses are summarised in Table 3.

Table 3: Gene copy number determination of DBT418 corn using Southern blot analysis

	Approximate gene copy number	
Region	**Intact**	**Rearranged**
cryIAc	2	0
bar	1	1
pinII	0	0.5
adhI intron I	0	0.5
bla	4	0.5
ColE1	4	0

PCR analysis was then used to further characterise the inserted DNA in corn event DBT418. Numerous overlapping, long PCR products (4.8 – 13.0 Kb) were generated which spanned the length of the DNA insert in event DBT418. The PCR fragments were analysed with restriction enzymes and this data was used to construct a map of the

inserted DNA. To further define the 5' and 3' ends of the inserted DNA, the relevant PCR fragments were sub cloned and sequenced and this information was used to complete the map of the inserted DNA (see Figure 1).

The PCR and sequencing analysis confirmed the estimations of the gene copy number by Southern blot analysis (Table 3) although the more detailed information indicated that there are three, rather than four copies of the *bla* gene and ColE1 origin of replication.

PCR analysis was also used to further characterise the *pinII* insertion in DBT418 corn, which according to the Southern blot analysis was not present as a fully intact copy. A 370 bp PCR product spanning the *pinII* region in the DBT418 insertion site was cloned and then sequenced. Analysis of the DNA sequence confirmed that the *pinII* gene in DBT418 corn is not intact. The sequence analysis of this region indicated there are two potential open reading frames (ORFs), of 94 and 104 amino acids, which overlap the region corresponding to the *pinII* sequence and which are present on the two complementary DNA strands. To confirm that these ORFs are not expressed, Northern blot analysis was done using total RNA isolated from DBT418 leaves and kernels. The RNA was probed with a sequence specific to the *pinII* gene. No hybridisation signals were observed indicating that the two ORFs are unlikely to be expressed.

Conclusion

The molecular analyses indicate that transformation event DBT418 contains two copies of the *cryIAc* expression cassette, one functional copy of the *bar* expression cassette, three copies of the *bla* gene and the ColE1 origin of replication, plus several non-functional partial fragments of the *bar* and *pinII* genes all at the one insertion site. The evidence strongly indicates that the partial copy of the *pinII* gene is not expressed.

Stability of genetic changes

Studies evaluated:

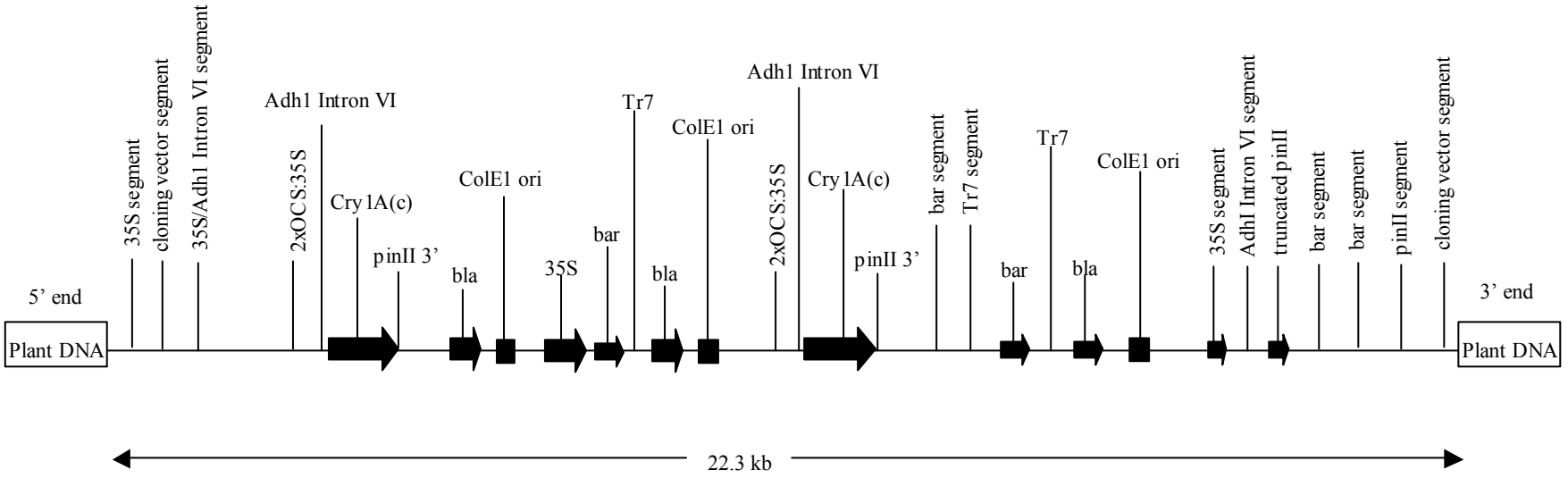
Stephens, M. *et al* (1996). Molecular characterization of transgene content and stability in transgenic corn hybrid line DK.DL (DBT418). Performing laboratory: DEKALB Genetics Corporation. Study No. DGC-95-A07.

Walters, D. (1996). Demonstration of stable Mendelian inheritance of *cryIAc* and *bar* genes in DBT418. Performing laboratory: DEKALB Genetics Corporation. Study No. DGC-95-A14.

Southern blot analysis

As a demonstration of genetic stability, a large number of DBT418 plants were analysed using Southern blot analysis to determine the frequency at which variations in *cryIAc* content occurred. Non-segregating hybrid seed, produced by crossing a female elite inbred (DK) homozygous for the DBT418 event with a non-transformed male inbred (DL), were planted in the field and leaf tissue was collected from 190 of these plants.

Figure 1: Map of inserted DNA in event DBT418



Out of the samples analysed, the vast majority exhibited the expected Southern hybridisation pattern indicating that both copies of the *cryIAc* gene had been stably inherited. Only four out of the 190 plants analysed had a hybridisation pattern that was atypical. Three of these four plants contained a single copy of the *cryIAc* gene, i.e. had lost one copy of the *cryIAc* gene through normal genetic processes. The other plant had neither copy of the *cryIAc* gene. These plants were not further characterised. A low level of genetic variation is considered normal.

PCR analysis

DBT418 plants have been repeatedly backcrossed to non-transformed inbred plants to introgress the DBT418 event into elite inbred germplasm for the production of hybrids. Progeny of DBT418 plants that were backcrossed to non-transformed inbreds were tested to determine if the DBT418 event is inherited in a predictable manner consistent with that expected of a single nuclear genetic locus, that is, in a Mendelian manner. Progeny derived from a cross between a parent hemizygous for the DBT418 event and a non-transformed parent would be expected to contain the DBT418 event at a frequency of about 50% (that is, the ratio of transgenic to non-transgenic should be 1:1).

One early generation backcross population and three late generation backcross populations were analysed for segregation of the *cryIAc* and *bar* genes using PCR. The genotypes tested are listed in Table 4.

Table 4: DBT418 and control genotypes tested by PCR

Genotype code	Genotype designation	No. of crosses
AW/BC5/DBT418	Late generation	6
BS/BC5/(AW.DBT418)	Late generation	7
DK/BC6/(AW.DBT418)	Late generation	8
DBT418(AW)08(aBK)	Early generation	2
AW	Non-transformed control	-

Approximately 100 seeds per DBT418 genotype and 50 seeds from the non-transformed control were planted and grown using standard methods for propagation of corn in a greenhouse. Samples of leaf tissue for PCR analysis were taken from young seedlings at 12 to 13 days post planting. Samples were taken from about 48 seedlings of each DBT418 genotype and 24 control seedlings. PCR analysis was subsequently performed on 43 samples from each set of samples. A non-transformed control was analysed with each set of 43 samples of a given genotype.

Chi-square analysis was done to assess the hypothesis that segregation is Mendelian (that is, occurs at a ratio of 1:1), as would be expected from a single genetic locus in a cross involving a transformed hemizygote and a non-transformed individual. The hypothesis that segregation occurred in a 1:1 ratio was accepted if chi-square values gave a probability of 5% (P value of 0.05) or greater.

The extracted DNA was analysed for *cryIAc*, *bar* and an endogenous maize gene, *adh* (coding for alcohol dehydrogenase), which serves as an internal control. Included in each set of PCR reactions for each genotype were two positive control reactions

containing the relevant plasmid DNA. Two negative control reactions, containing no DNA, were included with each set of PCR reactions.

The results of the PCR analyses are summarised in Table 5.

Table 5: Results of segregation analysis using PCR

Genotype	No. of plants tested	Gene tested	No. of PCR positive plants	No. of PCR negative plants	χ^2	P
AW/BC5/DBT418	43	*cryIAc*	22	21	0	0.95
		bar	22	21	0	0.95
		adh	43	0		
BS/BC5/(AW.DBT418)	43	*cryIAc*	24	19	0.37	0.50
		bar	24	19	0.37	0.50
		adh	43	0		
DK/BC6/(AW.DBT418)	43	*cryIAc*	24	19	0.37	0.50
		bar	24	19	0.37	0.50
		adh	43	0		
DBT418(AW)08(aBK)	43	*cryIAc*	20	23	0.10	0.70
		bar	19	24	0.37	0.50
		adh	43	0		
AW	5	*cryIAc*	0	5		
		bar	0	5		
		adh	5	0		

In all the genotypes tested, *bar* and *cryIAc* appear to segregate together in plants in approximately a 1:1 ratio to plants that lack the two genes.

Conclusion

The *bar* and *cryIAc* genes in DBT418 corn are tightly linked and segregate together in a Mendelian fashion suggesting that both genes are inserted at the same genomic location. This further supports the results of the molecular characterisation. In the vast majority of cases, both genes are stably maintained in the corn genome through several generations of backcrosses.

Antibiotic resistance genes

Antibiotic resistance genes can be present in some transgenic plants as a result of their use as marker genes to select transformed cells. It is generally accepted that there are no safety concerns with regard to the presence in the food of antibiotic resistance gene DNA *per se* (WHO 1993). However, concerns have been expressed that there could be horizontal gene transfer of antibiotic resistance genes from ingested food to microorganisms present in the human digestive tract and that this could compromise the therapeutic use of antibiotics.

This section of the report will therefore concentrate on evaluating the human health impact of the potential transfer of antibiotic resistance genes from insect-tolerant and

glufosinate ammonium tolerant corn to microorganisms present in the human digestive tract.

In the DBT418 corn lines, PCR analysis demonstrated that DBT418 corn contains three copies of the *bla* gene under the control of a bacterial promoter. The *bla* gene encodes the enzyme β-lactamase and confers resistance to a number of β-lactam antibiotics such as penicillin and ampicillin. The *bla* gene is not expressed in DBT418 corn.

The first issue that must be considered in relation to the presence of an intact *bla* gene in DBT418 corn is the probability that this gene would be successfully transferred to and expressed in microorganisms present in the human digestive tract. The following steps are necessary for this to occur:

- excision of DNA fragments containing the *bla* gene and its bacterial promoter;
- survival of DNA fragments containing the *bla* gene in the digestive tract;
- natural transformation of bacteria inhabiting the digestive tract. This requires the recipient cells to be physiologically competent (competence depends on growth conditions, the age of the cells and environmental conditions) (Stewart and Carlson 1986);
- survival of the bacterial restriction system by the DNA fragment containing the *bla* gene;
- stable integration of the DNA fragment containing the *bla* gene into the bacterial chromosome or plasmid; and
- maintenance and expression of the *bla* gene by the bacteria.

The transfer of a functional *bla* gene to microorganisms in the human digestive tract is therefore considered highly unlikely because of the number and complexity of the steps that would need to take place consecutively. It should also be noted that the processing steps for corn typically include heat, solvent or acid treatments that would be expected to remove and destroy DNA. Intact fragments of the *bla* gene are unlikely to survive the processing steps making the chance of horizontal gene transfer even more unlikely. The processing steps can also lead to the release of cellular enzymes (nucleases) that are responsible for degrading DNA into smaller fragments.

The second and most important issue that must be considered is the potential impact on human health in the unlikely event that successful transfer of a functional *bla* gene to microorganisms in the human digestive tract occurred.

In the case of transfer of the *bla* gene from DBT418 corn to microorganisms of the digestive tract, the human health impacts are considered to be negligible. This is because ampicillin-resistant bacteria are commonly found in the digestive tract of healthy individuals (Calva *et al* 1996) as well as diseased patients (Neu 1992).

Therefore, the additive effect of a *bla* gene from DBT418 corn being taken up and expressed by microorganisms of the human digestive tract would be insignificant compared to the population of ampicillin resistant bacteria already naturally present.

In relation to considering the potential impact on human health from the transfer of other novel genetic material to human cells via the digestive tract, it is important to note that humans have always consumed large amounts of DNA as a normal component of food and there is no evidence that this consumption has had any adverse effect on human health. Furthermore, current scientific knowledge has not revealed any DNA sequences from ingested foods that have been incorporated into human DNA. Novel DNA sequences in genetically modified foods comprise only a minute fraction of the total DNA in the food (generally less than 0.01%) and are therefore unlikely to pose any special additional risks compared with the large amount of DNA naturally present in all foods.

Conclusion

It is extremely unlikely that the ampicillin resistance gene or other novel genetic material will transfer from foods derived from DBT418 corn to bacteria or other cells in the human digestive tract because of the number and complexity of steps that would need to take place consecutively. In the highly unlikely event that the resistance gene was transferred the human health impacts would be negligible because ampicillin-resistant bacteria are already commonly found in the human gut and in the environment.

The probable degradation and removal of DNA through the processing steps for corn further mitigate against any horizontal transfer of DNA from DBT418 corn to cells in the human digestive tract.

CHARACTERISATION OF NOVEL PROTEIN

Biochemical function and phenotypic effects

On the basis of the molecular and phenotypic characterisation, corn containing the DBT418 event would be expected to express two new proteins – a truncated form of the insecticidal protein CryIAc and PAT. No β-lactamase expression would be expected in DBT418 corn, as the *bla* gene does not have the appropriate regulatory sequences for plant expression. Expression of the potato protease inhibitor II would also not be expected because the *pinII* expression cassette is not intact.

CryIAc

The synthetic *cryIAc* gene encodes the CryIAc protein of 613 amino acids with a predicted molecular weight of 66 kDa

Like other insecticidal crystal proteins produced by *Bacillus thuringiensis*, CryIAc is naturally produced in the bacterium as a 130 kDa protoxin that is cleaved by trypsin in the insect gut to a tryptic core protein of approximately 66 kDa. It is this 66 kDa trypsin resistant core that is toxic to susceptible lepidopteran larvae (Bietlot *et al* 1989, Hofte

and Whitely 1989). The trypsin resistant core is essentially composed of the amino-terminal half of the protein excluding the first 28 amino acids of the protoxin which are also cleaved off by trypsin. The CryIAc coding sequence introduced into DBT418 corn only differs from the native trypsin resistant core fragment in that the 28 amino acids at the amino-terminus have been retained.

Studies evaluated:

Millham, R.D. *et al* (1996). Characterization of the Cry1Ac protein from transgenic plants and demonstration of equivalence to microbially produced Cry1Ac. Performing laboratory: DEKALB Genetics Corporation. Study No.DGC-95-A19.

In this study CryIAc protein isolated from DBT418 plants was analysed to assess its equivalence to CryIAc protein purified from *Bacillus thuringiensis* — characteristics analysed were molecular weight, immunogenicity, amino-terminal sequence, insecticidal activity and glycosylation.

The DBT418 plant used for this study had the genotype AW/BC2/DBT418.BS/BC1/DBT418 (2Bt). The non-transformed control plant was of the DK.DL genotype. Leaf tissue was collected from more than 50 plants (field and glasshouse grown) and then pooled into a single sample so that adequate protein could be extracted for the analyses.

The apparent molecular weights and immunogenicity of the DBT418 corn and *B. thuringiensis* produced CryIAc proteins were compared using Western blot analysis. A single immunogenic protein band of approximately 66 kDa was detected in extracts from both DBT418 corn and *B. thuringiensis*. As expected, no such band was seen in the control samples.

The CryIAc protein encoded by the DBT418 event contains six potential glycosylation sites however as the encoded protein has not been specifically targeted to the endoplasmic reticulum where glycosylation occurs, no glycosylation would be expected. A glycoprotein detection assay confirmed that CryIAc expressed in DBT418 corn had not been glycosylated.

Amino-terminal sequencing of the native CryIAc protein yielded a match with the predicted sequence of the trypsinised CryIAc core fragment from which the first 28 amino acids have been cleaved, leaving Ile_{29} of the protoxin sequence as the terminal residue. Amino-terminal sequencing of the CryIAc from DBT418 corn also yielded a sequence that is an exact match with amino acid residues 26 through 33 of the CryIAc coding sequence of the native *Bacillus cryIAc* gene.

This indicates that the amino-terminus of CryIAc extracted from DBT418 begins at amino acid Gly_{26}, which is three residues longer at the amino-terminus than the native CryIAc. It is not known if the removal of the first 25 amino acids of the DBT418 CryIAc occurs *in vivo* or whether it occurred during the purification of the protein for analysis.

The functional activity of the DBT418 plant CryIAc protein was evaluated using tobacco hornworm bioassays on lyophilised leaf material. Results from these assays demonstrated nearly 100% mortality of insects in a 5-day assay.

Conclusion

The native and DBT418 CryIAc are identical in their electrophoretic mobility and immunogenicity and are virtually identical in their amino-terminal sequence. There was no evidence of glycosylation of either the DBT418-derived or native CryIAc. The DBT418 corn derived CryIAc is also insecticidally active.

Phosphinothricin acetyl transferase

PAT is a protein consisting of 183 amino acids with a molecular weight of about 22 kDa. The PAT enzyme catalyses the transfer of an acetyl group from acetyl CoA to the amino group of phosphinothricin. The enzyme is highly substrate specific (Thompson *et al* 1987). The substrate affinity for phosphinothricin is more than 30 times higher than affinity for demethylphosphinothricin (the biosynthetic pathway intermediate) and over 300 times higher than affinity for the amino acid glutamate. Therefore as its affinity for related PPT compounds is very low and no additional substrates have ever been reported it is highly unlikely that any naturally occurring compounds in corn would react with PAT.

Study evaluated:

Laccetti LB, Adams WR, Nutkis JE, Millham RD and Walters DS (1996). Characterization of the phosphinothricin acetyltransferase protein from transgenic plants and demonstration of equivalence to microbially produced phosphinothricin acetyltransferase. Performing laboratory: DEKALB Genetics Corporation. Study No.DGC-95-A20.

In this study PAT protein isolated from DBT418 plants was analysed to assess its equivalence to PAT protein purified from *Escherichia coli* — characteristics analysed were molecular weight, immunogenicity, amino-terminal sequence, enzyme activity and glycosylation.

Leaf tissue collected from field and glasshouse grown AW/BC2/DBT418.BS/BC1/DBT418 (2Bt) and DK.DL lines was used as the source of test and control proteins for analysis. The reference protein was a microbial produced PAT containing an amino-terminal extension of 20 amino acids, including six histidine residues (a His-tag) and a thrombin protease cleavage site. The His-tag facilitates the one-step purification of the protein using affinity chromatography with nickel resin. The majority of this extension can subsequently be removed from the affinity-purified protein through treatment with thrombin protease, leaving a protein with only four additional amino acids at its amino-terminus.

The apparent molecular weights and immunogenicity of the plant and microbial produced PAT proteins were compared using Western blot analysis with antibodies specific to PAT. An immunogenic PAT band of approximately 23 kDa was clearly present in tissue from DBT418 plants. No such band was present in the extract from

control plants. In the lane in which the microbial produced PAT was run a band of approximately 25 kDa was detected. This molecular weight is consistent with what would be expected given that the microbial produced PAT has an amino-terminal extension that adds an additional 1.9 kDa to the molecular weight of the protein. Elimination of the His-tag by thrombin cleavage produced a band having a similar electrophoretic mobility as the DBT418 PAT.

Results of glycoprotein detection assays showed, as expected, no evidence of glycosylation of either DBT418 PAT or microbial PAT.

Amino-terminal sequence data was obtained for DBT418 PAT, microbial PAT with the His-Tag intact and thrombin-treated microbial PAT. These experimentally determined sequences were compared to the deduced animo acid sequence from the *bar* gene inserted into DBT418 corn. The experimentally determined animo-terminal sequence of the DBT418 PAT protein matched with the deduced amino acid sequence of the *bar* gene used to transform the corn. The animo terminal sequence of the His-Tag PAT is identical to that of the DBT418 plant PAT except for the presence of the His-Tag. The thrombin-treated His-Tag PAT is identical DBT418 PAT except for the four additional amino-terminal residues – glycine, serine, histidine, and methionine.

The enzymatic activity of DBT418 PAT was compared to microbial His-Tag PAT. The PAT activity of the His-Tag PAT was similar to, although slightly lower than, that observed for DBT418 PAT. This result demonstrates that the addition of the His-Tag to PAT has not altered the essential characteristics of the enzyme, as expressed in DBT418 corn.

Conclusion

The DBT418 plants produce a single PAT protein of the expected molecular weight that is recognised by PAT antibodies. The DBT418 PAT and microbial-produced PAT exhibited similar electrophoretic mobility, immunogenicity and enzymatic activity, and their amino terminal amino acid sequence matched the deduced amino acid sequence derived from the *bar* gene used to transform corn and *E. coli*. Neither the DBT418 nor microbial PAT showed any evidence of glycosylation.

Protein expression analysis

Study evaluated:

Kruger, D.E. *et al* (1996). Magnitude of transgenic protein accumulation in transformed DBT418 corn lines. Performing laboratory: DEKALB Genetics Corporation, Mystic. Study No. DGC-95-A01.

The genotypic backgrounds containing the DBT418 insertion event and control genotypes that were analysed in this study are detailed in Table 6.

Table 6: Genotypic backgrounds used for protein level determinations

Genotype	Abbreviation	DBT418 allele	Description
AW/BC2/DBT418 S4	S4 inbred	Segregating	Unfinished inbred
AW/BC2/DBT418.BS/BC1/DBT418(2Bt)	2Bt hybrid	Homozygous	Unfinished hybrid
DK.DL(DBT418)	DK.DL(DBT418)	Hemizygous	Finished hybrid
DK.DL	DK.DL	None	Control hybrid
AW	AW	None	Control hybrid

Tissue samples were collected from three field locations during the 1995 growing season. Each field study site consisted of one plot with thirteen rows, each row with a separate corn line (either a DBT418 line or a control line). Tissue samples were collected at five distinct time points over the course of the growing season and the samples analysed to determine the concentration of the novel proteins. The tissue sampled at the various time points were:

(a) leaf and root tissue from the V6-V7 growth stage (collar of the 6 or 7th true leaf is visible);
(b) leaf, stalk, root, ball, pollen, silk, whole plant including root of the pollen shedding stage;
(c) whole plant not including roots from the dough stage (typical for silage corn);
(d) leaf, stalk, root ball, and ear (husk removed) of the harvest stage; and
(e) whole plant including roots of the senescence stage.

The data relating to the kernel are the most important as the kernel is the only part of the plant used for human consumption.

CryIAc

An enzyme-linked immunosorbent assay (ELISA) was used to quantify the CryIAc protein in the various tissue samples. For each DBT418 tissue type analysed for CryIAc expression, control non-transformed plants were also analysed. No CryIAc expression was detected in any of the control genotypes analysed.

The protein expression data indicates that the pattern of expression is similar in all three genotypes analysed. The 2Bt hybrid (homozygote) generally exhibited higher levels of CryIAc expression than the hemizygote hybrid (DK.DL(DBT418). An exception to this pattern was observed in kernel tissue where both lines produced similar levels of CryIAc.

The highest tissue expression levels were found in the leaves with the highest levels occurring at the harvest stage. The levels of CryIAc in kernels was generally low but detectable at the time of harvest. Mean levels ranged from 36.0 to 42.8ng/g dry weight for the three genotypes. This is equivalent to about 0.0001% of the total kernel protein.

A summary of the CryIAc expression data is presented in Table 7. The data for the kernel expression is highlighted.

Table 7: CryIAc protein levels during DBT418 corn development

Tissue	Genotype	Mean protein levels (ng/g dry weight [n; SE])		
		V6-V7	Pollen shed	Harvest
Leaf:	S4 inbred	217.9 (7; 46.23)	335.0 (8; 74.93)	459.6 (8; 99.84)
	DK.DL(DBT418)	177.8 (8; 42.22)	93.7 (8; 7.39)	620.6 (8; 84.39)
	2Bt hybrid	289.6 (4; 36.74)	174.2 (4; 36.69)	1198.4 (4; 270.78)
Stalk:	S4 inbred	N/A	28.5[c] (3;4.19)	123.6[a] (7; 46.72)
	DK.DL(DBT418)	N/A	BLD[*] (8)	40.9 (8; 7.34)
	2Bt hybrid	N/A	BLD (4)	115.1 (4; 25.19)
Root ball:	S4 inbred	69.8 (7; 17.34)	78.2 (8; 12.56)	58.7 (8; 25.19)
	DK.DL(DBT418)	50.9 (8; 9.58)	57.7[a] (7; 16.24)	58.0[b] (5; 8.22)
	2Bt hybrid	117.9 (4; 17.08)	72.0 (4; 20.56)	125.4 (4; 16.93)
Kernel:	S4 inbred	N/A	N/A	42.8 (6; 16.60)
	DK.DL(DBT418)	N/A	N/A	37.1 (8; 3.97)
	2Bt hybrid	N/A	N/A	36.0 (4; 8.14)
Silk:	S4 inbred	N/A	BLD (8)	N/A
	DK.DL(DBT418)	N/A	110.5[d] (2; 10.70)	N/A
	2Bt hybrid	N/A	BLD (4)	N/A
Pollen:	1Bt hybrid[#]	N/A	BLD (8)	N/A
	DK.DL(DBT418)	N/A	BLD (4)	N/A
	2Bt hybrid	N/A	BLD (8)	N/A
Whole plant:	S4 inbred	N/A	147.1 (8; 47.87)	N/A
	DK.DL(DBT418)	N/A	35.9 (8; 5.44)	N/A
	2Bt hybrid	N/A	75.0 (4; 15.03)	N/A

[*] BLD below the limit of detection of the assay (6.7 ng/g dry weight)
[a] 1 of 8 samples were BLD, [b] 3 of 8 samples were BLD, [c] 5 of 8 samples were BLD, [d] 6 of 8 samples were BLD
[#] AW/BC2/DBT418.BS/BC1/DBT418 (1Bt) genotype was substituted for the S4 hybrid because insufficient pollen was available from the S4 hybrid.

PAT protein

A quantitative immunoblot was used to determine the quantity of PAT protein, using an enhanced chemiluminescence system in conjunction with scanning densitometry.

The tissue distribution for PAT expression was similar to that found for CryIAc except that levels of PAT were significantly higher. Leaf tissue is the site of highest PAT expression in DBT418 plants with means for the three genotypes ranging from 501.8 to 1099.4µg/g dry weight at the pollen shed stage. Relatively low levels of PAT were found in the kernel, mean levels for the three genotypes ranging from 3.1 to 6.0µg/g dry weight. This is equivalent to about 0.0175% of the total kernel protein.

In many of the tissues analysed the PAT levels found in the homozygous line were approximately double the levels found in the hemizygous line. This would be expected if expression levels were additive based on the number of DBT418 events present. In general, the genetic background was not found to exert a great deal of influence on PAT expression levels, with all three genotypes examined expressing similar protein levels characteristic for the particular tissue type

A summary of the PAT expression data is presented in Table 8. The data for kernel expression is highlighted.

Table 8: PAT protein levels during DBT418 corn development

Tissue	Genotype	Mean protein levels (µg/g dry weight [n; SE])		
		V6-V7	Pollen shed	Harvest
Leaf:	S4 inbred	351.1 (7; 52.91)	522.0 (6; 59.04)	60.8[a] (6; 12.46)
	DK.DL(DBT418)	276.3 (8; 25.51)	501.8 (8; 34.75)	180.5 (8; 24.68)
	2Bt hybrid	554.9 (2; 136.03)	1099.4 (3; 76.29)	213.6 (4; 61.92)
Stalk:	S4 inbred	N/A	75.8 (8; 12.24)	95.2 (6; 16.86)
	DK.DL(DBT418)	N/A	60.0 (8; 11.98)	64.4 (8; 8.23)
	2Bt hybrid	N/A	77.0 (4; 11.66)	136.3 (2; 12.74)
Root ball:	S4 inbred	95.1 (7; 16.91)	54.1 (8; 9.15)	24.5 (7; 3.71)
	DK.DL(DBT418)	59.4 (8; 3.53)	27.5 (8; 6.25)	21.3 (8; 2.23)
	2Bt hybrid	88.1 (4; 21.45)	69.5 (4; 23.58)	28.8 (3; 7.37)
Kernel:	S4 inbred	N/A	N/A	6.0 (6; 1.88)
	DK.DL(DBT418)	N/A	N/A	3.1 (8; 0.35)
	2Bt hybrid	N/A	N/A	4.9 (4; 0.63)
Silk:	S4 inbred	N/A	128.2 (8; 17.21)	N/A
	DK.DL(DBT418)	N/A	29.1 (8; 2.97)	N/A
	2Bt hybrid	N/A	133.3 (2: 60.01)	N/A
Pollen:	1Bt hybrid[#]	N/A	BLD[*] (8)	N/A
	DK.DL(DBT418)	N/A	BLD (8)	N/A
	2Bt hybrid	N/A	BLD (4)	N/A
Whole plant:	S4 inbred	N/A	111.1 (8; 16.50)	N/A
	DK.DL(DBT418)	N/A	72.8 (8; 5.88)	N/A
	2Bt hybrid	N/A	119.5 (4; 25.63)	N/A

[*] BLD below the limit of detection of the assay (12.10 µg/g dry weight)
[a] 2 of 8 samples were BLD and not used to calculate the mean or standard error
[#] AW/BC2/DBT418.BS/BC1/DBT418 (1Bt) genotype was substituted for the S4 hybrid because insufficient pollen was available from the S4 hybrid.

PIN II protein

DBT418 corn does not contain an intact copy of the *pinII* gene and is therefore not expected to produce the serine protease inhibitor. Consequently, the PIN II protein analysis was done as a qualitative assay only to determine the presence or absence of the protein in a variety of DBT418 plant tissues.

The three plant genotypes analysed were the same as those analysed for CryIAc and PAT expression. The limit of detection of the PIN II immunoblot assay for most tissues (leaf, stalk and root) is 400ng/g dry weight. The results of the PIN II protein expression analysis are summarised in Table 9.

Table 9: Summary of PIN II analysis in various lyophilised DBT418 tissues

Tissue	Growth stage	No. of genotypes evaluated	Total No. of DBT418 plants analysed	Assay limit of detection (per g dry weight)	PIN II detection
Leaf	V6-V7	3	10	400 ng	ND
Leaf	Pollen shed	3	10	400 ng	ND
Stalk	Pollen shed	3	6	400 ng	ND
Root	Pollen shed	3	6	400 ng	ND
Pollen	Pollen shed	3	6	Indeterminate	ND
Kernel	Harvest	3	10	1800 ng	ND

No evidence was found for the presence of PIN II in any of the tissues analysed. Comparison of control lanes with DBT418 lanes on the immunoblot revealed no additional immunogenic bands that were not present in the control extracts.

β-lactamase

Several copies of the *bla* gene are present in DBT418 corn. As the *bla* gene is under the control of a bacterial promoter it should not be expressed in DBT418 corn. To determine whether DBT418 corn produced any ß-lactamase, plant tissue samples were assayed using an immunoblot for the presence of the enzyme in a variety of DBT418 tissues. The DBT418 genotypes analysed were the same as for the previous protein expression analyses discussed above. The limit of detection of the ß-lactamase immunoblot was less than 9 µg/g dry weight.

No evidence was found of expression of ß-lactamase protein in DBT418 corn. Comparison of control lanes with DBT418 lanes also revealed no additional immunogenic bands in the DBT418 lane that were not present in the control extracts.

Conclusion

CryIAc and PAT protein expression was detected in several tissue types and throughout plant development in three different genetic backgrounds containing the DBT418 event. PAT was expressed at significantly higher levels than CryIAc in the corresponding tissues in which it was detected. The highest protein expression levels were in leaf, with significantly less protein being expressed in kernels. In kernels, mean CryIAc levels ranged from 36.0 – 42.8ng/g dry weight (equivalent to about 0.0001% of the total protein) and mean PAT levels ranged from 3.1 – 6.0µg/g dry weight (equivalent to about 0.0175% of the total protein). In general, the different genetic backgrounds did not appear to influence protein expression levels, which appeared to be more greatly influenced by tissue type. No evidence for either PIN II or ß-lactamase expression was found in any of the DBT418 plants tested.

Potential toxicity of novel proteins

The potential toxicity of the CryIAc and PAT proteins was evaluated using acute oral toxicity in mice and in birds. The scientific basis for using an acute test is that, if toxic, proteins are known to act via acute mechanisms and laboratory animals have been shown to exhibit acute toxic effects from exposure to proteins known to be toxic to humans (Sjoblad *et al* 1992). Bacterial proteins produced by fermentation, rather than proteins purified from the transgenic plants, were used for the acute toxicity studies in mice, because of the difficulty of obtaining sufficient material from plants. As described in Section 3.2, microbial produced Cry1Ac and PAT were shown to be equivalent to the transgenic proteins produced by DBT418 corn.

Studies evaluated:

Merriman, T.N. (1996). An acute oral toxicity study in mice with *Bacillus thuringiensis* subsp *kurstaki* Cry1Ac delta endotoxin. Performing laboratory: DEKALB Genetics Corporation and Springborn Laboratories (SLI). Study No. DEKALB – DGC-95-A17 / SLI No. 3406.1.

Merriman, T.N. (1996). An acute oral toxicity study in mice with phosphinothricin acetyltransferase (PAT) protein. Performing laboratory: DEKALB Genetics Corporation, Springborn Laboratories (SLI). Study No. DEKALB – DGC-95-A18 / SLI No. 3406.2.

Palmer, S.J. and Beavers, J.B. (1996). Lyophilized DBT418 leaf tissue: a dietary toxicity study with the northern bobwhite. Performing laboratory: DEKALB Genetics Corporation. Study No. DGC-95-A13.

CryIAc – acute toxicity study in mice

CryIAc is insecticidal only to lepidopteran insects (MacIntosh *et al* 1990b) and its specificity of action is directly attributable to the presence of specific receptors in the target insects (Wolfersberger 1990, Ferré *et al* 1991). There are no receptors for the δ-endotoxins of *B. thuringiensis*, including CryIAc, on the surface of mammalian intestinal cells (Hofmann *et al* 1988; MacIntosh *et al* 1990b).

Young adult CD-1(ICR)BR mice (source: Charles River Laboratories, Portage, MI) were acclimatised for at least 5 days before dosing. They were housed individually in controlled conditions with free access to food and water, except for the 3–4 hours before dosing, when food was withheld. *Bacillus thuringiensis* Cry1Ac delta-endotoxin (lot CSV-102695, purity 66.5%) was administered to the mice (5/sex) at 5000 mg/kg bodyweight (bw) in a volume of 20 mL/kg bw by single oral gavage, equivalent to 3825 mg of Cry1Ac protein.

Mice were observed for clinical signs twice on the day of dosing (post dosing) and once daily after this for the 14-day duration of the test. Bodyweight was determined before fasting and before dosing on day 0 and on days 7 and 14. At the end of the study, mice were killed and examined for gross pathology. Any abnormalities were recorded.

There was one death on day 1 due to gavage error. No deaths and no clinical abnormalities were observed in the 9 remaining mice. The LD_{50} was determined to be >3825 mg/kg bw in mice.

PAT – acute toxicity study in mice

Young adult CD-1(ICR)BR mice (source: Charles River Laboratories, Portage, MI) were acclimatised for at least 5 days before dosing. They were housed individually in controlled conditions with free access to food and water, except for the 3–4 hours before dosing, when food was withheld. Histidine-tagged PAT protein (lot CSV-102695, purity >99%) was administered to the mice (5/sex) at 2500 mg/kg bodyweight (bw) in a volume of 20 mL/kg bw by single oral gavage.

Mice were observed for clinical signs three times on the day of dosing (post dosing) and once daily after this for the 14-day duration of the test. Bodyweight was determined before fasting and before dosing on day 0, and on days 7 and 14. At the end of the

study, mice were killed and examined for gross pathology. Any abnormalities were recorded.

There were no deaths during the study. The only clinical abnormality observed was few faeces in one male. During the 7–14-day interval one male had a slight loss of bodyweight, the 9 other mice gained weight. No gross intestinal findings were seen on day 14. The LD_{50} was determined to be >2500 mg/kg bw in mice.

CryIAc and PAT protein – avian toxicity study

Northern bobwhite quails (14 days of age, source: Wildlife International Ltd) were acclimatised from day of hatch until initiation of testing. Each treatment or control group was made up of 10 birds uniquely identified by wing tags. The birds used in the study were immature and could not be differentiated by sex. Birds were housed in controlled conditions with free access to food and water during acclimatisation and during the test. The applicant provided test and control corn leaf protein to the testing laboratory. The control lyophilised corn leaf material was received in three shipments (lots 3495, 3504 and 3507) and contained 0% CryIAc protein, while the test lyophilised corn leaf material was received in two shipments (lots 3496 and 3505). The DBT418 lyophilised corn leaf material contained CryIAc protein at 150.5 ng/g dry weight, determined by ELISA, and PAT protein at 209.8 µg/g dry weight, determined by protein immunoblotting.

Three replicate groups, each containing 10 chicks, received a diet containing lyophilised DBT418 leaf tissue at 200 000 parts per million (ppm) or 20% weight per weight (w/w). One control group containing 10 chicks received lyophilised control leaf tissue at 200 000 ppm or 20% w/w. Another control group received untreated diet only. For the test, each group was fed the appropriate test or control diet for five days and then given untreated feed for three days.

During acclimatisation, all birds were observed daily. Birds exhibiting abnormal behaviour or physical injury were not used for the test. Throughout the test, all birds were observed at least twice daily. A record was maintained of all mortality, signs of toxicity and abnormal behaviour. Bodyweight was measured at the initiation of the test, at the end of the exposure period on day 5 and at termination of the test on day 8. Average feed consumption was determined for each group for days 0–5, and days 6–8.

No birds died during the test period and there were no abnormal clinical signs or behavioural changes in any group. There were no treatment-related effects on bodyweight or food consumption during this study. The dietary LC_{50} for northern bobwhite exposed to lyophilised DBT418 leaf tissue in the diet was determined to be greater than 200 000 ppm or 20% w/w.

Conclusion

The results do not indicate any potential toxicity from either the Cry1Ac protein or PAT.

Potential allergenicity of novel protein

The concerns regarding potential allergenicity of novel proteins are two fold. Firstly, there are concerns that the ability to express new or different proteins in food will result in the transfer of allergens from one food to another, thereby causing some individuals to develop allergic reactions to food they have not previously been allergic to. Secondly, there are concerns that the transfer of novel proteins to food will lead to the development of new allergies in certain individuals. The former is more easily addressed than the latter because if an allergen is already known it is possible, using human sera or human skin tests, to test if it has been transferred. There are no reliable tests or animal models, however, which enable the prediction of the allergenic potential of novel proteins. Instead, potential allergenicity can only be indicated by examination of a number of characteristics of the novel protein, such as whether it is derived from a known allergenic source, its physical/chemical characteristics (most allergens have a molecular mass between 10 and 70 kDa, are glycosylated, and are resistant to acid and protease degradation), whether it has any sequence similarity to any known allergens, and whether it is likely to be present in large amounts in the food as consumed and therefore have potential for allergic sensitisation.

Studies evaluated:

Walters, D.S. and Adams, W. (1996). *In vitro* digestibility of CryIAc and PAT proteins. Performing laboratory: DEKALB Genetics Corporation. Study No. DGC-96-A22.

Appendix 2: CryIAc and phosphinothricin acetyl transferase proteins show no homology to allergenic proteins. (Appendices to submission to ANZFA for the inclusion of corn containing the DBT418 gene by Monsanto in Standard A18 – Food derived from gene technology.)

Digestibility of CryIAc and PAT

E. coli produced CryIAc and PAT as well as PAT protein purified directly from DBT418 corn were subject to digestion under simulated gastric conditions. The microbial produced CryIAc protein had been experimentally determined to be equivalent to that which is expressed in DBT418 plants. The bacterially produced proteins were used to spike protein extracts from leaf tissue from non-transgenic plants. The proteins were added to leaf extract to give a concentration of 500 ng CryIAc/5 µL extract and 1900 ng PAT/5 µL extract.

The extracts were added to simulated gastric fluid (SGF) containing 3.2 mg/mL pepsin (1X), no pepsin or a 100 fold dilution of pepsin (0.01X). Samples were taken at 0, 2, 5, 15 and 30 minutes and analysed using immunoblotting.

CryIAc degraded rapidly in 1X SGF. No trace of CryIAc was detectable by immunoblot at 0 or 2 minutes. CryIAc was not degraded in SGF lacking pepsin. In 0.01X SGF, significant degradation of CryIAc was seen at 0 and 2 minutes incubation, and after 5 minutes no CryIAc protein could be detected.

PAT protein also degraded rapidly in 1X SGF. Significant degradation occurred at 0 minutes, and after 2 minutes only trace amounts of PAT protein were detectable. No degradation was seen in SGF lacking pepsin.

In 0.01X SGF, significant degradation of PAT was seen at 0 and 2 minutes incubation, and after 5 minutes no PAT protein could be detected. DBT418 leaf protein extract was also added to 0.01X SGF. PAT was visible at the time 0 point but was not detectable after 2 minutes incubation in 0.01X SGF.

The results demonstrate that both CryIAc and PAT are digested as normal dietary protein, both being rapidly degraded in the proteolytic and acid conditions of simulated gastric fluid suggesting they would not survive mammalian digestion.

Comparison of CryIAc and PAT amino acid sequence with known allergens

The amino acid sequences of CryIAc and PAT proteins in DBT418 corn and those of known allergens were compared. A significant sequence similarity was defined as a sequence identity of eight or more contiguous amino acids. A database of known allergenic proteins was assembled from the public domain genetic databases including GenPept, PIR and SwissProt. After eliminating duplicated and irrelevant sequences, a database of 276 known allergens remained. The database was searched for sequences similar to CryIAc and PAT proteins using the program FASTA.

The search did not identify any allergens with significant amino acid sequence similarity to either CryIAc or PAT.

Conclusion

As CryIAc and PAT are present at very low levels in the kernel, are easily digested in conditions mimicking mammalian digestion and do not show any significant amino acid sequence similarity with known allergens, they have limited potential to become a food allergen.

COMPARATIVE ANALYSES

Key nutrients

There are concerns that genetic modification will affect the overall nutritional composition of a food, or cause unintended changes that could adversely affect the safety of the product. Therefore a safety assessment of food produced from transgenic plants must include analysis of the composition of the food, based on a comparison with other commercial varieties of the crop. Generally, comparisons are made not only with the parental line but also with other non-transformed lines. If the parameter for the transformed line is within the normal range for non-transformed lines, this is considered acceptable (Hammond and Fuchs 1998).

To determine whether unexpected changes had occurred in the nutritional composition of corn as a result of the DBT418 insertion event, and to assess the nutritional adequacy

of the corn, compositional analyses of forage and grain were undertaken. Material was collected for analysis from DTB418 corn grown in field trials in the United States in 1995. The genotype tested was the same as used for the molecular characterisation and the protein expression analyses – that is, DK.DL(DBT418). This hybrid was sold commercially as DK566-DBT418. A non-transformed version of the same hybrid was used as a control.

Major components of the grain and forage (proximates) were determined using conventional chemical methods (Association of Official Analytical Chemists, AOAC; and American Oil Chemists Society, AOCS) and near infrared transmission (NIT) spectroscopy. Analysis included protein, oil, fibre, ash, starch and moisture. Chemical determination of composition was determined on samples collected from ten locations. NIT data was derived from samples collected from nine locations. Amino acid composition of DBT418 and control grain was determined by acid hydrolysis of corn meal and reverse phase high-performance liquid chromatography. Fatty acid composition was determined by trans-esterification and gas chromatography. Nine samples, representing three locations, were tested, except for fatty acid analysis where only three samples, representing one location, were tested.

Results are shown in Tables 10 – 13.

Table 10: Proximate analysis[1] of grain from DBT418 corn

Constituent analysed	Chemical analysis[2]		NIT analysis[3]		Literature Range[4]
	DBT418	control	DBT418	control	
Protein	9.02 ± 0.22	8.56 ± 0.16	9.06 ± 0.19	9.09 ± 0.17	6.0 – 12.0
Oil	4.05 ± 0.05	3.92 ± 0.04	4.16 ± 0.04	4.12 ± 0.04	3.1 – 5.7
Fibre	1.96 ± 0.03	2.02 ± 0.03	Not done	Not done	2.0 – 5.5
Ash	1.32 ± 0.01	1.30 ± 0.02	Not done	Not done	1.1 – 3.9
Moisture	8.14 ± 0.04	8.22 ± 0.04	70.61 ± 0.20	70.62 ± 0.17	7 – 23
Starch	Not done	Not done	5.63[5] ± 0.17	5.57[5] ± 0.11	-

[1] values are expressed as a % (dry weight basis) and are the mean ± S.E.
[2] sample size of 30
[3] sample size of 27
[4] Watson 1987
[5] samples were artificially dried

The proximate composition of grain from DBT418 corn is equivalent to that of the control hybrid lacking the DBT418 insertion event and the levels are also comparable to the available literature ranges for these constituents.

Table 11: Proximate analysis[1] of forage from DBT418 plants

Constituent	DBT418 hybrid[2]	Control[3]	Literature range[4]
Protein	6.81 ± 0.23	7.12 ± 0.29	3.5 – 15.9
Oil	2.77 ± 0.07	2.82 ± 0.06	0.7 – 6.7
Fibre	20.56 ± 0.03	20.57 ± 0.38	-
Ash	4.33 ± 0.15	4.28 ± 0.13	1.3 – 10.5
Moisture	66.68 ± 0.04	66.96 ± 0.04	-

[1] values are expressed as a % (dry weight basis) and are the mean ± S.E.
[2] sample size = 24
[3] sample size = 30
[4] Watson 1987

No significant differences were observed in proximate composition of forage between the DBT418 hybrid line and the control hybrid line. The proximate levels were also comparable to the literature reported range, where these were available.

Table 12: Amino acid composition of grain from DBT418 and control hybrids

	mg amino acid / g dry weight	
Amino acid[1]	**DBT418 hybrid**	**Control hybrid**
Aspartate + asparagine	2.58 ± 0.14	2.29 ± 0.15
Glutamate + glutamine	12.95 ± 0.64	13.75 ± 0.54
Serine	2.84 ± 0.26	2.99 ± 0.32
Glycine	3.08 ± 0.06	3.12 ± 0.06
Threonine	2.11 ± 0.09	2.1 ± 0.11
Arginine	3.95 ± 017	3.98 ± 0.21
Alanine	4.59 ± 0.17	4.85 ± 0.13
Valine	3.14 ± 0.11	3.22 ± 0.12
Phenylalanine	3.19 ± 0.16	3.43 ± 0.14
Isoleucine	2.39 ± 0.09	2.46 ± 0.10
Leucine	7.33 ± 0.42	7.91 ± 0.34
Lysine	2.68 ± 0.06	2.70 ± 0.06

[1] Tryptophan, cysteine and proline are acid labile therefore no values are reported. Methionine, histidine and tyrosine levels were not determined.

No significant differences in the amino acid composition of grain were observed between the DBT418 hybrid line and the control hybrid line.

Table 13: Fatty acid composition of grain from DBT418 and control hybrids

	% Total fatty acids		
Fatty acid[1]	**DBT418 hybrid**[2]	**Control hybrid**[3]	**Literature Range**[4]
Palmitic (16:0)	13.8	13.7	7 - 19
Stearic (18:0)	4.1	4.0	1 - 3
Oleic (18:1)	27.2	28.2	20 - 46
Linoleic (18:2)	53.4	52.6	35 - 70
Linolenic (18:3)	1.2	1.2	0.8 – 2.0
Eicosenoic (20:0)	0.5	0.6	

[1] Other fatty acids were below the limit of detection.
[2] The values for DBT418 corn are the means of three samples from one location.
[3] The values for the control are the means of nine samples, three from each of three locations.
[4] Watson 1982.

No significant differences in the fatty acid composition of grain were observed between the DBT418 hybrid line and the control hybrid line.

Compositional analysis of commercial hybrids

In addition to the above data, compositional data was also generated during the period of product use, 1997-1999, prior to the discontinued marketing of the product beginning in 2000. This data was generated from the analysis of commercial hybrid corn seed lots, archived material, and field samples. In these analyses, a number of hybrids, including all five hybrids that were sold commercially in 1999, were analysed for nutritional composition including proximate, amino acid content and calcium and phosphorus content. The results of these analyses are presented in Tables 14 – 16.

No major differences in proximate of grain were observed between the commercial DBT418 hybrids line and the control hybrids. The values reported were comparable to the literature reported ranges.

No significant differences in amino acid content of grain were observed between the commercial DBT418 hybrids line and the control hybrids. Except for tyrosine, the values reported were comparable to the literature reported ranges. The values for tyrosine for both the DBT418 and control hybrids were low compared to the literature reported ranges.

No meaningful differences were observed between the DBT418 and control hybrids. The values for phosphorus were also comparable to the literature reported values. The values for calcium, for both the DBT418 hybrids and the control hybrids, were low when compared to the literature reported values. This is most likely due to environmental factors and is not considered to be treatment related.

Conclusion

Grain from hybrid corn lines containing the DBT418 event is compositionally no different to grain from hybrid corn lacking the DBT418 event.

Table 14: Proximate analysis[1] of commercial F1 DBT418 and control hybrid corn seed

	DBT418 hybrids[2]						
	DK493-DBT418	**DK566-DBT418**	**DK580-DBT418**	**DK595-DBT418**	**DK626-DBT418**	**Control hybrids[3]**	**Literature values[4]**
Protein	9.8 (9.7-10.0)	9.6 (9.1-9.9)	9.5 (9.2-10.2)	10.4 (10.0-10.9)	11.7 (11.5-12.0)	10.2 (9.0-11.8)	9.5 (6.0-12.0)
Oil	4.1 (4.0-4.2)	4.8 (4.8-4.9)	4.2 (4.1-4.3)	3.9 (3.8-4.0)	4.4 (4.2-4.5)	4.2 (3.7-4.9)	4.3 (3.1-5.7)
Ash	1.3 (1.3-1.3)	1.3 (1.2-1.3)	1.3 (1.2-1.3)	1.3 (1.3-1.4)	1.3 (1.3-1.3)	1.3 (0.5-1.5)	1.4 (1.1-3.9)
Fibre	2.7 (2.6-2.8)	2.6 (2.4-2.8)	4.2 (3.9-4.3)	3.1 (2.7-3.5)	3.0 (2.8-3.2)	3.0 (2.0-4.1)	3.3 (3.3-4.3)
Moisture	11.1 (10.6-11.8)	11.9 (11.7-12.1)	11.9 (11.6-12.1)	11.4 (11.4-11.4)	11.7 (11.5-11.9)	11.6 (10.7-12.6)	(7 – 23)

[1] Mean and (range) reported as percent on a dry weight basis (except for moisture)
[2] Values derived from three lots tested for each hybrid (n=3) except for DK595-DBT418 (n=2).
[3] Analysis based on three lots each of DK493, DK566, DK580, DK595, and DK626. Values for individual controls were also provided by the applicant.
[4] Values for grain purchased off the open market (Watson 1987).

Table 15: Amino acid analysis[1] of commercial F1 DBT418 and control hybrid corn seed

	DBT418 hybrids[2]						
	DK493-DBT418	**DK566-DBT418**	**DK580-DBT418**	**DK595-DBT418**	**DK626-DBT418**	**Control hybrids[3]**	**Literature values[4]**
Lysine	2.8 (2.8.3.0)	2.7 (2.7-2.7)	2.9 (2.6-3.1)	2.5 (2.4-2.6)	2.3 (2.2-2.3)	2.7 (2.3-3.1)	2.5 (2.0-3.8)
Threonine	3.1 (3.0-3.1)	3.1 (3.1-3.2)	3.6 (3.4-3.7)	3.3 (3.2-3.4)	3.0 (3.0-3.0)	3.4 (2.9-3.7)	3.8 (2.9-3.9)
Isoleucine	2.6 (2.6-2.7)	2.7 (2.6-2.7)	2.7 (2.6-2.8)	2.7 (2.7-2.8)	2.7 (2.7-2.7)	2.8 (2.5-3.2)	4.2 (2.6-4.0)
Histidine	2.5 (2.5-2.6)	2.5 (2.4-2.5)	2.5 (2.3-2.6)	2.4 (2.4-2.5)	2.3 (2.2-2.3)	2.5 (2.2-2.7)	2.1 (2.0-2.8)
Valine	3.7 (3.7-3.8)	3.7 (3.5-3.7)	3.7 (3.5-3.8)	3.7 (3.7-3.8)	3.5 (3.4-3.6)	3.8 (3.4-4.3)	4.7 (2.1-5.2)
Leucine	10.7 (10.5-10.8)	11.2 (11.0-11.3)	11.1 (11.0-11.4)	12.2 (12.0-12.3)	12.2 (12.0-12.5)	11.3 (10.4-12.6)	11.2 (7.8-15.2)
Arginine	3.8 (3.7-3.9)	3.7 (3.6-3.7)	4.0 (3.7-4.1)	3.5 (3.4-3.6)	3.2 (3.1-3.4)	3.6 (3.2-4.2)	5.8 (2.9-5.9)
Phenylalanine	4.1 (4.1-4.2)	4.3 (4.2-4.4)	4.5 (4.4-4.5)	4.6 (4.5-4.6)	4.6 (4.5-4.7)	4.3 (4.1-4.7)	4.9 (2.9-5.7)
Glycine	3.7 (3.6-3.7)	3.6 (3.5-3.7)	3.6 (3.3-3.8)	3.4 (3.2-3.5)	3.3 (3.2-3.4)	3.5 (3.2-3.8)	3.7 (2.6-4.7)
Alanine	6.9 (6.8-7.0)	7.2 (7.1-7.2)	6.9 (6.8-7.0)	7.4 (7.4-7.4)	7.4 (7.3-7.5)	7.0 (6.6-7.4)	7.8 (6.4-9.9)
Aspartic acid	6.6 (6.6-6.7)	6.7 (6.7-6.7)	6.7 (6.5-6.9)	6.6 (6.4-6.8)	6.2 (6.1-6.2)	6.5 (6.0-6.9)	6.8 (5.8-7.2)
Glutamic acid	16.3 (16.1-16.5)	17.0 (16.9-17.0)	17.4 (17.2-17.8)	18.1 (17.7-18.5)	17.3 (17.0-17.6)	19.2 (16.6-24.3)	17.7 (12.4-19.6)
Proline	8.3 (8.0-8.5)	8.6 (8.5-8.7)	8.0 (7.7-8.2)	8.8 (8.8-8.8)	9.1 (8.9-9.1)	8.5 (8.1-9.1)	8.4 (6.6-10.3)
Serine	5.6 (5.6-5.7)	5.9 (5.8-5.9)	5.1 (5.1-5.1)	5.5 (5.1-5.9)	5.9 (5.8-6.0)	5.2 (4.5-5.9)	4.6 (4.2-5.5)
Tyrosine	1.7 (1.6-1.7)	1.7 (1.7-1.8)	1.7 (1.6-1.8)	1.8 (1.7-1.8)	1.6 (1.7-1.8)	1.7 (1.5-1.8)	4.7 (2.9-4.7)

[1] Mean and (range) reported as percent total protein. Analyses for methionine, cystine and tryptophan not included.
[2] Values derived from three lots tested for each hybrid (n=3) except for DK595-DBT418 (n=2).
[3] Analysis based on three lots each of DK493, DK566, DK580, DK595, and DK626. Values for individual controls were also provided by the applicant.
[4] Values for grain (Watson 1982).

Table 16: Calcium and phosphorus content[1] of commercial F1 DBT418 and control hybrid corn seed[2]

	Hybrid						
	DK493-DBT418	**DK493**	**DK595-DBT418**	**DK595**	**DK626-DBT418**	**DK626**	**Literature[3]**
Ca	ND-0.007	ND-0.004	ND-0.003	ND-0.003	0.003 (0.003-0.003)	0.003 (0.003-0.003)	0.03 (0.01-0.1)
P	0.26 (0.24-0.27)	0.26 (0.25-0.28)	0.29 (0.29-0.30)	0.25 (0.24-0.26)	0.25 (0.23-0.27)	0.31 (0.28-0.32)	0.27 (0.26-0.75)

[1] Mean and (range) reported as percent total calcium or phosphorus.
[2] Phosphorus and calcium content were determined through analysis of randomly chosen archived commercial F1 hybrid seed lots. Because the samples were not drawn from a controlled trial, the results are not suitable for statistical analysis.
[3] Values for grain purchased from the open market (Watson 1982).

Key toxicants

There are no naturally occurring toxins known to occur at biologically significant levels in corn (Wright 1987).

Key anti-nutrients

Corn contains few natural toxins or anti-nutrients. The anti-nutrients trypsin and chymotrypsin inhibitors are present in corn at very low levels and are not considered nutritionally significant (Wright 1987).

Naturally occurring allergenic proteins

Corn does not contain any known naturally occurring allergenic proteins.

NUTRITIONAL IMPACT

In assessing the safety of food produced using gene technology, a key factor is the need to establish that the food is nutritionally adequate and will support typical growth and well-being. In most cases, this can be achieved through an understanding of the genetic modification and its consequences, together with an extensive compositional analysis of the food.

Where, on the basis of available data, there is still concern or doubt in this regard, carefully designed feeding studies in animals may provide further reassurance that the food is nutritionally adequate. Such studies may be considered necessary where the compositional analysis indicates significant differences in a number of important components or nutrients, or where there is concern that the bioavailability of key nutrients may be compromised by the nature of the genetic changes to the food.

In the case of DBT418 corn, the extent of the compositional and other data provided in this application is considered adequate to establish the safety of the food.

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