FOOD DERIVED FROM INSECT-PROTECTED, GLUFOSINATE AMMONIUM-TOLERANT CORN LINE DAS-59122-7

A SAFETY ASSESSMENT

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SUMMARY

Food derived from genetically modified (GM) corn line DAS-59122-7 has been assessed for its safety for human consumption. This corn line has been genetically modified to be resistant to insect attack and herbicide tolerant and has been developed for cultivation in North America.

A number of criteria have been addressed in the safety assessment including: a characterisation of the transferred genes, their origin, function and stability; changes at the DNA, protein and whole food levels; compositional analyses; evaluation of intended and unintended changes; and the potential for the newly expressed proteins to be either allergenic or toxic to humans.

History of Use

Corn (*Zea mays L)*, otherwise known as maize, is the world's third leading cereal crop, behind wheat and rice, and is grown in over 25 countries worldwide. Corn-derived products are routinely used in a large number and diverse range of foods and have a long history of safe use. Products derived from DAS-59122-7 corn may include flour, breakfast cereals, high fructose corn syrup and other starch products.

Description of the Genetic Modification

Corn line DAS-59122-7 contains two novel genes, *cry34Ab1* and *cry35Ab1,* encoding the insecticidal proteins Cry34Ab1 and Cry35Ab1. These two genes were derived from the soil bacterium *Bacillus thuringiensis* and are selectively toxic to certain insect pests of corn. Corn line DAS-59122-7 also contains a copy of the *pat* gene, encoding the enzyme phosphinothricin acetyl transferase (PAT), which confers tolerance to the herbicide glufosinate ammonium.

Detailed molecular and genetic analyses of corn line DAS-59122-7 indicate that the transferred *cry34Ab1*, *cry35Ab1* and *pat* genes are stably integrated into the plant genome at one insertion site and are stably inherited from one generation to the next.

Characterisation of Novel Protein

Corn line DAS-59122-7 expresses three novel proteins – Cry34Ab1, Cry35Ab1, and PAT. In the corn grain, the PAT protein is undetectable. Cry34Ab1 is expressed at levels ranging from 28.9-117 ng/mg dry weight in DAS-59122-7 corn grain and Cry35Ab1 at levels ranging from not detectable to 1.83 ng/mg.

Acute oral toxicity studies have been conducted on the Cry34Ab1, Cry35Ab1, and PAT proteins – there was no evidence of toxicity in all cases. Potential allergenicity was assessed by sequence comparison to known allergens, simulated digestion studies and by determining thermolability – these data did not indicate any potential for allergenicity.

Comparative Analyses

Compositional analyses were done to establish the nutritional adequacy of grain from corn line DAS-59122-7, and to compare it to a non-transgenic control line and commercial varieties of corn. The constituents measured were protein, fat, carbohydrate, ash, moisture, fibre, fatty acids, amino acids, vitamins, minerals, secondary metabolites and anti-nutrients.

No differences of biological significance were observed between the transgenic corn grain and its non-GM counterpart. Several minor differences in key nutrients and other constituents were noted however the levels observed represented very small differences and do not indicate an overall pattern of change that would warrant further investigation. On the whole, it was concluded that food from corn line DAS-59122-7 is equivalent in composition to that from other commercial corn varieties.

Nutritional Impact

The detailed compositional studies are considered adequate to establish the nutritional adequacy of the food and indicate that food derived from corn line DAS-59122-7 is equivalent in composition to food from non-GM corn varieties. The introduction of food produced from corn line DAS-59122-7 into the food supply is therefore expected to have minimal nutritional impact.

Conclusion

No potential public health and safety concerns have been identified in the assessment of food produced from corn line DAS-59122-7. On the basis of the available data, food produced from corn line DAS-59122-7 can be considered as safe and as wholesome as food produced from other corn varieties.

BACKGROUND

A safety assessment has been conducted on food derived from a new genetically modified (GM) corn variety. The GM corn variety is known as DAS-59122-7 corn. No commercial name had been defined at the time of the assessment.

Corn line DAS-59122-7 has been genetically modified for protection against the Western corn rootworm (*Diabrotica vigifera*), Northern corn rootworm (*Diabrotica berberi*), and Mexican corn rootworm (*Diabrotica vigifera zeae*). These species are serious insect pests of dent corn in the major corn-producing states of the north-central United States and Canada. Protection is conferred by the expression in the plant of bacterially derived protein toxins (*Bt*-δ-endotoxins) that are specific for these insects. Corn line DAS-59122-7 also contains a gene encoding resistance to the herbicide glufosinate ammonium.

Corn line DAS-59122-7 contains three novel genes, *cry34Ab1*, *cry35Ab1*, and *pat*. The two *cry* genes express insecticidal crystal proteins and the *pat* gene expresses the enzyme phosphinothricin acetyltransferase (PAT) which confers tolerance to the herbicide glufosinate ammonium.

Commercial corn lines containing the *cry* genes from *Bacillus thuringiensis* (*Bt*) will provide growers with effective methods for controlling corn rootworm. *Bt* formulations are widely used as biopesticides on a variety of cereal and vegetable crops grown organically or under conventional agricultural conditions.

Corn, together with rice and wheat, is one of the most important cereal crops in the world with total production of 591 million tonnes in 2000 (FAO, 2001). The majority of grain and forage derived from maize is used in animal feed. Maize grain is also used in industrial products, such as ethyl alcohol by fermentation and highly refined starch by wet-milling.

Domestic production of corn in Australia and New Zealand is supplemented by the import of a small amount of corn-based products, largely as high-fructose corn syrup, which is not currently manufactured in either Australia or New Zealand. Such products are processed into breakfast cereals, baking products, extruded confectionery and corn chips. Other corn products such as cornstarch are also imported and used by the food industry for the manufacture of dessert mixes and canned foods.

Corn line DAS-59122-7 is permitted for food and feed use in the United States. Corn line DAS-59122-7 is not being developed for cultivation in Australia. Therefore, if approved, food from corn line DAS-59122-7 may enter the Australian and New Zealand food supply as imported food products.

HISTORY OF USE

Donor Organisms

Bacillus thuringiensis

The source of the *cry34Ab1* and *cry35Ab1* genes used in this GM corn is the ubiquitous soil and plant bacterium *Bacillus thuringiensis* (*Bt*). Both *cry* genes are synthetic versions of genes from the non-motile strain of *Bt*, PS149B1.

The WHO International Program on Chemical Safety (IPCS) report on environmental health criteria for *Bt* concludes that '*Bt* has not been documented to cause any adverse effects on human health when present in drinking water or food' (IPCS, 1999).

Bt proteins are used widely as an insecticide in both conventional and organic agriculture. In Australia, various *Bt* insecticidal products are registered with the Australian Pesticides and Veterinary Medicines Authority (APVMA) for use on cotton, vegetables, fruits, vines, oilseeds, cereal grains, herbs, tobacco, ornamentals, forestry and turf. The very wide use of formulations containing the *Bt* insecticidal proteins indicates that people eating and handling fresh foods are commonly in contact with this protein.

Insecticidal products using *Bt* were first commercialised in France in the late 1930s (Nester et al 2002) and were first registered for use in the United States by the Environment Protection Agency (EPA) in 1961 (EPA, 1998). The EPA thus has a vast historical toxicological database for *B. thuringiensis*, which indicates that no adverse health effects have been demonstrated in mammals in any infectivity/ pathogenicity/ toxicity study (McClintock *et al*., 1995; EPA, 1998; Betz *et al*., 2000). This confirms the long history of safe use of *Bt* formulations in general, and the safety of *B. thuringiensis* as a donor organism.

Streptomyces viridochromogenes

Streptomyces viridochromogenes is a ubiquitous soil fungus and was the source of the PAT encoding gene that is present in corn line DAS-59122-7. *S. viridochromogenes* is a gram positive sporulating soil bacteria. Few *Streptomyces* have been isolated from animal or human sources and pathogenicity is not a typical property of these organisms. *S. viridochromogenes* is itself not known to be a human pathogen and nor has it been associated with other properties (e.g. production of toxins) known to affect human health.

Agrobacterium tumefaciens

The species *Agrobacterium tumefaciens* is a Gram-negative, non-spore forming, rod-shaped bacterium commonly found in the soil. It is closely related to other soil bacteria involved in nitrogen fixation by certain plants.

Agrobacterium naturally contains a plasmid (the *Ti* plasmid) with the ability to enter plant cells and insert a portion of its genome into plant chromosomes. Normally therefore, *Agrobacterium* is a plant pathogen causing root deformation mainly with sugar beets, pome fruit and viniculture crops. However, adaptation of this natural process has now resulted in the ability to transform a broad range of plant species without causing adverse effects in the host plant.

Other donor organisms

The regulatory elements that were used in the gene construct were derived from *Solanum tuberosum* (potato), *Triticum aestivum* (wheat) and *Zea mays* (corn), plants that are widely consumed and generally recognised as safe. CaMV 35S promoter and terminator sequences are frequently used in transgenic plants and have no pathological characteristics (USDA, 1995).

Host Organism

Corn (*Zea mays L)*, otherwise known as maize, is the world's third leading cereal crop, behind wheat and rice, and is grown in over 25 countries worldwide (OECD, 2002b). Worldwide production of maize is 500 million tons a year, with the United States and China being the major producers.

The majority of grain and forage derived from maize is used as animal feed, however maize also has a long history of safe use as food for human consumption. The grain can be processed into industrial products such as ethyl alcohol (by fermentation), and highly refined starch (by wet-milling) to produce starch and sweetener products. In addition to milling, the maize germ can be processed to obtain corn oil and numerous other products (White and Pollak, 1995).

Corn plants usually reproduce sexually by wind-pollination. This provides for natural out-crossing between plants, but it also presents an opportunity for plant breeders to produce hybrid seed by controlling the pollination process. Open pollination of hybrids in the field leads to the production of grain with properties derived from different lines and, if planted, would produce lower yields (CFIA, 1994). Instead, by controlling the cross-pollination of inbred lines from chosen genetic pools (using conventional techniques), the combining of desired genetic traits into a controlled hybrid line results in improved agronomic performance and increased yields. This inbred-hybrid concept and resulting yield response is the basis of the modern seed industry in several food commodities including corn.

The commercial production of corn has seen many improvements, particularly since the 1920's when corn varieties were developed by conventional breeding between progeny of two inbred lines to give hybrid varieties that were known to be superior to open-pollinated varieties in terms of their agronomic characteristics. In present agricultural systems, hybrid corn varieties are used in most developed countries for consistency of performance and production.

The corn recipient line was the public line designated Hi-II. Hi-II is a derivative of the A188 and B73 inbred lines of corn which are publicly available inbred lines from the University of Minnesota and Iowa State University, respectively. Hi-II is approximately 50:50 of the two lines (Armstrong *et al.*, 1991).

DESCRIPTION OF THE GENETIC MODIFICATION

Method used in the genetic modification

Studies submitted

Coats, I. and Herman, R. (2002) Product Characterisation Data for *Bacillus thuringiensis* Cry34Ab1 and Cry35Ab1 Proteins Expressed in Transgenic Maize Plants (PHP17662). Pioneer Hi-bred International, Johnston, Iowa. Study ID: PHI-2002-046

Coats, I. and Herman, R. (2003) Addendum to MRID#45790601: Product Characterisation Data for *Bacillus thuringiensis* Cry34Ab1 and Cry35Ab1 Proteins Expressed in Transgenic Maize Plants (PHP17662). Pioneer Hi-bred International, Johnston, Iowa. Study ID: PHI-2002-046

Corn line DAS-59122-7 was produced by *Agrobacterium*-mediated transformation of *Zea mays line* Hi-II, using the transformation vector PHP17662. The plasmid contains the *cry34Ab1*, *cry35Ab1*, and *pat* genes and regulatory elements as shown in Table 1.

Immature embryos of corn were treated with *Agrobacterium tumefaciens* strain LBA4404 containing plasmid PHP17662. After a period of embryo and *Agrobacterium* co-cultivation on solid culture medium, the embryos were transferred to fresh culture medium that contained the herbicide glufosinate ammonium. The culture medium was stimulatory to the maize somatic embryogenesis and was selective for those cells that contain the integrated *pat* gene. The embryonic tissue was then regenerated into whole transgenic plants, which were transferred to the greenhouse.

Leaf samples were taken for molecular analysis to verify the presence of the transgenes by PCR and to confirm the expression of the *cry* proteins by ELISA. Plants were also subjected to a whole plant bioassay using corn rootworm. Positive plants were crossed with an inbred line to obtain seed from the initially transformed plants. A number of lines were evaluated in the field which resulted in the selection of line DAS-59122-7, based on its good agronomic characteristics and excellent resistance to corn rootworm.

Table 1: Genetic elements of the plasmid PHP17662

Genetic element	Size (bp)	Function
Right border	25	T-DNA right border region
UBI1ZM PRO	1,986	Ubiquitin promoter (plus ubiquiting 5' untranslated region and intron) from Zea mays (Christensen *et al.*, 1992).
cry34Ab1	369	Synthetic version of the *cry34Ab1* gene encoding the 14 kDa delta-endotoxin parasporal crystal protein from Bt (maize optimised).
PINII TERM	1,299	Terminator sequence from Solanum tuberosum proteinase inhibitor II (An *et al.*, 1989).
TA PEROXIDASE	1,299	Root-preferred promoter from Triticum aestivum peroxidase (Hertig *et al.*, 1991).
cry35Ab1	1,152	Synthetic version of the *cry35Ab1* gene encoding a 44 kDa delta endotoxin parasporal crystal protein from Bt (maize optimised).
PINII TERM	318	Terminator sequence from Solanum tuberosum proteinase inhibitor II (An *et al.,* 1989).
CaMV 35S PRO	549	35S promoter from the cauliflower mosaic virus, Strasbourg strain (Hohn *et al.*, 1982).
pat	552	Synthetic, plant optimised phosphinothrycin acetyltransferase coding sequence from *Streptomyces viridochromogenes*
CaMV 35S TERM	199	35S terminator from cauliflower mosaic virus
LEFT BORDER	25	T-DNA left border region

Function and regulation of novel genes

cry34Ab1 and cry35Ab1

The maize optimised synthetic *cry34Ab1* and *cry35Ab1* genes encode proteins 123 and 383 amino acids in length respectively. Although these genes were originally isolated from *B. thuringiensis*, the DNA sequences of these two genes have been modified in order to alter the guanosine and cytosine codon bias to a level more typical for plant codons. The deduced amino acid sequences of these proteins expressed in the transgenic corn are identical to the native Cry34Ab1 and Cry35Ab1 protein sequences. The regulatory elements are described in Table 1. The *cry34Ab1* gene is regulated by the ubiquitin promoter from *Zea mays* and the *Solanum tuberosum* proteinase inhibitor terminator. The *cry35Ab1* gene is regulated by the wheat peroxidase gene promoter and the *Solanum tuberosum* proteinase inhibitor terminator.

The *cry34Ab1* and *cry35Ab1* genes confer protection against corn rootworm. This is described in more detail in section 4.1.

Pat

The *pat* gene encodes the PAT enzyme, which confers resistance to the herbicide glufosinate ammonium. This gene was introduced as a selectable marker for the identification of transformed plants. The *pat* gene was originally isolated from *Streptomyces viridochromogenes* Tu494, but as with the two *cry* genes, in this construct the codons have been optimised for plant expression. The deduced amino acid sequence is identical to the native bacterial PAT enzyme.

The cauliflower mosaic virus 35S promoter controls the transcription of the *pat* gene in corn line DAS-59122-7.

No other genes were transferred to corn line DAS-59122-7.

Characterisation of the genes in the plant

Studies submitted:
Cressman, R.F., Luckring, A.K., Sanders, C.D., Hunt, S.L. and Locke, M.E. (2004). Insert and Border Sequence Characterisation of *B.t.* Cry34/35Ab1 Event DAS-59122-7. Pioneer Hi-Bred International, study ID: PHI-2002-037

Locke, M.E. and Igo, E. (2003). Characterisation of DNA Inserted into Transgenic Corn Events DAS-45216-6 and DAS-59122-7. Pioneer Hi-Bred International, study ID: PHI-2002-038

Locke, M.E., Dietrich, N. and Weber, N. (2003). Detailed Characterisation of DNA Inserted into Transgenic Corn Events DAS-45216-6 and DAS-59122-7. Pioneer Hi-Bred International, study ID: PHI-2002-041

Insert and copy number

Southern blot analysis was used to establish the integration pattern and determine copy number of the *cry34Ab1*, *cry35Ab1*, and *pat* genes and to confirm the absence of DNA sequence from outside the T-DNA borders of the transformation vector.

Southern blot analyses of four different generations (designated T_1S_1, T_1S_2, BC_1 and BC_2S_1; described in Table 2) of corn line DAS-59122-7 demonstrate that the insert in corn line DAS-59122-7 occurred as a simple integration of a single intact T-DNA from plasmid PHP17662. No plasmid backbone fragments were present as determined by Southern blot analyses. In addition, the results did not indicate that rearrangements of the T-DNA had occurred, as all internal restriction sites appeared to be intact and produced hybridising fragments of the expected size. Figure 1 shows the insert in DAS-59122-7 corn.

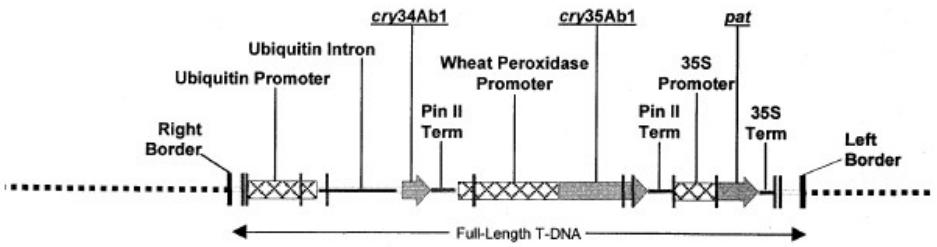

Figure 1: Schematic diagram of the DNA insert in corn line DAS-59122-7.

Table 2: Corn line DAS-59122-7 generations used in molecular characterisation studies

Generation	Description
T_0	Original Hi-II plant containing event DAS-59122-7
T_1S_1	T_0 generation corn plants were out-crossed for one generation to inbred line PH09B and selfed for one generation to produce the T_1S_1 seed
T_1S_2	T_0 generation corn plants were out-crossed for one generation to PH09B and selfed for two generations to produce the T_1S_2 seed
BC_1 hybrid	T_0 generation corn plants were out-crossed for one generation to inbred line PH09B. The resulting F_1 was crossed and then backcrossed to inbred 05F to make a BC_1. The BC_1 generation was then crossed to a second inbred 581 to produce the BC_1 hybrid seed
BC_2S_1	T_0 generation corn plants were out-crossed for one generation to PH09B, the resulting F_1 was crossed and then backcrossed twice to inbred 581 to make BC_2. The final generation represented here is a self-pollination (S_1) of the BC_2 creating a population that segregates at a ratio of 3:1.

PCR and sequence analysis

To further characterise the integrity of the inserted T-DNA and describe the genomic insertion site, the sequence of the T-DNA insert and flanking genomic DNA border regions of the insert in corn line DAS-59122-7 (T_1S_2) was determined. The entire insert was sequenced and this sequence compared to the DNA sequence of the transforming plasmid (PHP17662). In total, 7343 bp of T-DNA had become inserted into the corn genome. Twenty-two and 25 bp were found to be missing from the Right and Left border regions respectively. While T-DNA border sequences are known to play a critical role in T-DNA insertion into the genome, this result is not unexpected since insertions are often imperfect, particularly at the Left T-DNA border (Tinland and Hohn, 1995). Two nucleotide differences were observed in the non-translated wheat peroxidase promoter region of the T-DNA insert. Neither of these changes affected the open reading frame composition of the insert.

Flanking regions and putative Open Reading Frame analysis

The junctions between the insert and corn genomic regions were also sequenced. At the 5' end of the insert, 2593 bp of genomic DNA were sequenced, at the 3' end 1986 bp of genomic DNA were sequenced.

PCR amplification based on the insert and border sequences confirmed that the border regions were of maize origin. No further identification of the maize genomic border sequences was possible due to limited sequence homology with publicly available sequences in GenBank. Analysis of the sequence spanning the junction regions indicated that no novel open reading frame resulted from the insert in corn line DAS-59122-7.

Alignment of the entire transformation plasmid sequence with the border region sequences showed no significant homologies, indicating that the border regions do not contain fragments of the transforming plasmid.

The 5' and 3' junction regions between the corn genomic border sequence were analysed for the presence of novel open reading frames. No open reading frames of significant size (>100 amino acids) were identified in either region. The homology searches of these sequences with the known maize genomic sequences did not indicate the presence of endogenous maize open reading frames in the border regions that might have been disrupted by the insert in corn line DAS-59122-7.

Conclusion

Detailed molecular analyses have been performed on corn line DAS-59122-7 to characterise the novel genes present in the genome. Results indicate that there is one insertion site consisting of the entire T-DNA from plasmid PHP17662. The *cry34Ab1, cry35Ab1* and *pat* genes are intact.

Sequence analysis showed that two single nucleotide changes had occurred within the non-coding region of the insert. No novel ORFs (>100 amino acids) were created by the insertion of the novel genes and nor were any existing ORFs destroyed.

Stability of the genetic changes

Studies submitted:

Locke, M.E. and Igo, E. (2003). Characterisation of DNA Inserted into Transgenic Corn Events DAS-45216-6 and DAS-59122-7. Pioneer Hi-Bred International, study ID: PHI-2002-038

Locke, M.E., Dietrich, N. and Weber, N. (2003). Detailed Characterisation of DNA Inserted into Transgenic Corn Events DAS-45216-6 and DAS-59122-7. Pioneer Hi-Bred International, study ID: PHI-2002-041

Weber, N. and Igo E (2003) Characterisation of Transgenic Corn Event DAS-59122-7 to Investigate Genetic Equivalence of the Inserted DNA within a Single Generation. Pioneer Hi-Bred International, study ID: PHI-2003-012

Segregation analysis

Southern blot analysis was used to show that the insert is stably inherited within a single generation (Weber and Igo, 2003). Seventy-nine corn plants were grown from BC_2S_1 seed and were analysed for expression of the PAT (by leaf painting with glufosinate ammonium) and Cry34Ab1 (by lateral flow immunoassay) proteins. Of the 79 plants, 55 were positive for both PAT and Cry34Ab1 expression. The remaining 24 plants were negative for expression of both proteins (null segregants).

Genomic DNA was extracted from all 55 of the transgenic plants and 23 of the null segregants and used in Southern blotting to determine if the insert in each of the 55 plants was stably integrated. Southern blots were hybridised with probes specific to the *Cry34Ab1* gene, the *Cry35Ab1* gene, and the *pat* gene. The 23 null segregants showed no hybridisation with any of the three probes. The 55 transgenic plants all displayed a consistent hybridisation pattern with each of the probes, indicating the insert is the same in all individuals within the generation.

All results correlated with the previous Southern analyses on different generations of corn line DAS 59122-7 indicating that a single intact DNA insertion has integrated stably into the corn genome.

Chi squared analysis showed no significant difference between the observed ratio of 55 positive to 24 null plants in the BC_2S_1 generation to the expected segregation ratio of 3:1.

Another study analysed the Mendelian segregation of corn line DAS-59122-7 over eight generations. The T_0 generation corn plant was out-crossed for one generation to inbred line PH09B to produce T_1 generation plants which were either self pollinated to produce the T_1S_1 generation or out-crossed with Dow AgroSciences inbred lines designated inbred B (DAS male) or inbred C (DAS female) to produce a number of backcrosses. Since the insert in corn line DAS-59122-7 was expected to segregate as a single dominant gene, each generation was sprayed with glufosinate ammonium to eliminate susceptible plants to determine if the insert was segregating as expected.

All plants found to be herbicide tolerant were also tested with Cry34Ab1 immunoassay lateral flow devices. All of the plants determined to be herbicide tolerant were also positive for CryAb341. In five of the eight generations, no significant deviation from the expected segregation ratios was observed (Table 3).

Significant deviation from the expected segregation ratio occurred in the BC_1, BC_4 and BC_4S_1 generations in only one of two inbreds in each generation. A more consistent pattern of deviations from expected across generations and across inbred would be anticipated if the insert were responsible for these inconsistencies. The explanation given for the significant difference between the observed and expected segregation ratio in the BC_1 generation is the small sample size. A breeding error that allowed extra susceptible plants in the BC_4 and BC_2S_1 generations might also be an explanation. The deviation in the

BC_4 S_1 generation occurred only in one inbred background and was not seen in either inbred in the BC_2S_1 generation.

Since a majority of the generations showed no significant deviations from the expected ratios, and the deviations that occurred were inconsistent across generations and inbreds, it was concluded that the significant differences observed were likely to be due to experimental error and that the insert in corn line DAS-59122-7 is inherited as a Mendelian dominant gene.

A more powerful Chi-square test across all generations with an expected ratio of 1:1 (2644:2750) resulted in no significant difference between expected and observed ratios, as did a test across all generations with an expected segregation ratio or 3:1 (1354:472).

Table 3: Mendelian segregation of corn line DAS-59122-7

Generation	Expected segregation	Inbred	Number resistant	Number susceptible	Chi-Sq significance
T_1S_1	3:1	Hi-II	34	10	NS
F_1	1:1	Inbred B	21	23	NS
	1:1	Inbred C	22	28	NS
BC_1	1:1	Inbred B	57	80	P<0.05
	1:1	Inbred C	66	78	NS
BC_2	1:1	Inbred B	466	466	NS
	1:1	Inbred C	517	471	NS
BC_2S_1	3:1	Inbred B	267	82	NS
	3:1	Inbred C	302	98	NS
BC_3	1:1	Inbred B	431	434	NS
	1:1	Inbred C	415	447	NS
BC_4	1:1	Inbred B	451	483	NS
	1:1	Inbred C	198	240	P<0.05
BC_4S_1	3:1	Inbred B	369	121	NS
	3:1	Inbred C	382	161	P<0.025

Data expressed as the number of plants expected to be resistant to glufosinate ammonium : the number of plants expected to be susceptible.

Conclusion

The studies show that the T-DNA insert is stably integrated into the corn genome in line DAS-59122-7 and segregates as expected over the generations that were examined.

Antibiotic resistance genes

No antibiotic resistance marker genes are present in corn line DAS-59122-7.

CHARACTERISATION OF NOVEL PROTEINS

Biochemical function and phenotypic effects

Corn line DAS-59122-7 contains three novel proteins: Cry34Ab1; Cry35Ab1; and PAT.

Study submitted
Narva, K.E., Schnepf, H.E., Nygaard, L.R. and Wolt, J.D. (2003) Cry34/35 Protein Distribution and Familiarity. Regulatory Laboratories – Indianapolis Lab, Dow AgroSciences LLC Indiana. Study ID: GH-C 5702

Cry34Ab1 and Cry35Ab1

These proteins are insectidal δ-endotoxins derived from *B. thuringiensis* strain PS149B1. During sporulation, *B. thuringiensis* produces cytoplasmic inclusions containing one or more of the insecticidal crystal proteins. Most crystal proteins are synthesised intracellularly as inactive protoxins that spontaneously form small crystals, approximately 1 µm in size. Upon ingestion by susceptible insects, the highly alkaline pH of the midgut promotes solubilisation of the protoxin-containing crystals.

The protoxin is then activated by trypsin-like gut proteases, which cleave off domains from the carboxy- and amino- termini, leaving a protease resistant core, which is the active toxin. The active toxin binds to a highly specific glycoprotein receptor on the surface of midgut epithelial cells in the insect. Aggregation of the core toxins results in the 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 Whiteley, 1989; Schnepf *et al*., 1998).

Corn line DAS-59122-7 contains two separate parasporal crystal proteins, Cry34Ab1 (123 amino acids) and Cry35Ab1 (383 amino acids), with respective molecular weights of 14 kDa and 44 kDa. The transgenes that encode these proteins were optimised for expression in corn plants. The proteins encoded by the synthetic transgenes are identical in sequence to the native *B.t* crystal proteins.

The Cry34Ab1 and Cry35Ab1 proteins do not have a high degree of sequence homology to other Cry proteins currently in commercial transgenic plants. However, they are related to proteins present in commercial *Bt*-microbial products. Genomic serotyping of total genomic DNA from *B.t.* strain collections identified 78 strains containing sequences related to *cry35Ab1*. Crude fermentation broth extracts taken from a subsample of these strains showed the presence of one or both Cry34/35Ab1 proteins in 37 of 42 samples. Analysis of nucleic acid and deduced polypeptide sequences reveals that Cry34/35Ab1 proteins comprise large families of related insecticidal proteins.

Both proteins are required together for mortality of the corn rootworm larvae. Although the Cry34Ab1 protein is active alone in corn rootworm larvae when applied at high concentrations in bioassays, it appears that transgenic plants which express only the Cry34Ab1 protein do not control Western corn rootworms. The activity of the Cry34Ab1

protein in bioassays is greatly potentiated by Cry35Ab1. The Cry35Ab1 protein alone is not active against corn rootworm. *In vivo*, only a small quantity of Cry35Ab1 is needed in the Cry34/35Ab1 insecticidal crystal protein (ICP). Therefore, the majority of the activity seen with mixtures of Cry34Ab1 and Cry35Ab1 may be explained by the concentration of the Cry34Ab1 protein.

It is not known exactly how the Cry34/35Ab1 ICP exerts its toxicity. Histological studies have shown that the ICP causes disruption of the western corn rootworm larval midgut membranes. In experiments using artificial membranes, the ICP produces ion channels or pores which is at least partially responsible for the disruption of the synthetic membranes (Masson *et al.*, 2004). The formation of ion channels in artificial membranes has also recently been reported for Cry34Ab1 (Baum *et al.*, 2004). Meaningful *in vivo* activity with the ICP has only been observed in a subset of coleopteran larvae (corn rootworm). *In vivo* activity has not been found in adult corn rootworms, a corn aphid species or certain lepidopteran pests, indicating selective activity for corn rootworm larvae. Cry34Ab1 and Cry35Ab1 have not been observed to associate to form a hetero-dimer.

PAT

The herbicide tolerant trait, which was used as a selectable marker following transformation, is conferred by the expression of the introduced *pat* gene, which encodes the phosphinothricin acetyltransferase (PAT) protein.

The PAT protein consists of 183 amino acids, has a molecular weight of 22 kDa, and exhibits a high degree of enzyme specificity; recognising only one substrate. PAT functions by detoxifying phosphinothricin (PPT), the active constituent of glufosinate ammonium herbicides. PPT acts by inhibiting the endogenous enzyme glutamine synthetase, an enzyme involved in amino acid biosynthesis in plant cells. By inhibiting this enzyme, PPT causes rapid accumulation of ammonia in the plant cell, leading to plant death. In transformed corn plants, the introduced PAT enzyme chemically inactivates the PPT by acetylation of the free ammonia group, giving rise to herbicide tolerance in the whole plant.

Protein expression analysis

In corn line DAS-59122-7, it is expected that three novel proteins will be expressed. These are the Cry34Ab1, Cry35Ab1 and PAT proteins. Expression levels of these proteins were determined using enzyme-linked immunosorbent assay (ELISA) and are reported below.

Study submitted:
Essner, R (2003) Agronomic Characteristics, Quantitative ELISA and Nutrient Composition Analysis of Hybrid Maize Lines Containing the *cry34Ab1, cry35Ab1*, and *pat* Genes: Chile Locations. Pioneer Hi-Bred International Inc. Study ID PHI-2002-050

Field trials of corn line DAS-59122-7 and control lines were conducted in Chile in 2002-2003. Six separate field locations each contained four blocks. Each block contained the

corn line DAS-59122-7 hybrid[1] and a near isoline inbred control. Block 1 also contained the corn line DAS-59122-7 inbred[2]. Plots of the GM hybrids were either left untreated or received two sequential applications of a herbicide containing glufosinate ammonium as the active ingredient. Leaf, root, whole plant, pollen, stalk, forage, and grain samples were collected from the GM hybrid, GM inbred, and control lines and Cry34Ab1, Cry35Ab1 and PAT concentrations were measured using an ELISA.

No Cry34Ab1, Cry35Ab1 or PAT protein was detected in the control corn line. All matrices from DAS-59122-7 were found to express the Cry34Ab1 and Cry35Ab1 proteins at measurable levels. However, the PAT protein was undetectable in both the pollen and the grain of corn line DAS-59122-7. No PAT was detected in the forage samples from either of the hybrid DAS-59122-7 lines. The samples for the inbred DAS-59122-7 forage was pooled with other samples and not available for testing. All other matrices expressed PAT at detectable levels.

Expression levels of the three novel proteins in the corn grain are shown in Tables 5, 6, and 7. Mean expression levels of Cry34Ab1 in all matrices ranged from 29.2 ng/mg tissue dry weight (in sprayed hybrid DAS-59122-7 stalk) to 232 ng/mg tissue dry weight (in sprayed hybrid DAS-59122-7 leaf). Mean expression levels of Cry35Ab1 in all matrices ranged from 0.01 ng/mg tissue dry weight (in sprayed hybrid DAS-59122-7 pollen) to 85.3 ng/mg tissue dry weight (in non-sprayed hybrid DAS-59122-7 leaf). Mean expression levels of PAT ranged from below the limit of quantitation (LOQ) in pollen, forage and grain to 11.2 ng/mg tissue dry weight (in non-sprayed hybrid DAS-59122-7 leaf). These are shown in Table 4.

Table 4: Maximum and minimum mean expression levels of novel proteins in DAS-59122-7 corn

	Minimum Mean*	Maximum Mean*
Cry34Ab1	29.2 (sprayed hybrid stalk)	232 (sprayed hybrid leaf)
Cry35Ab1	0.01 (sprayed hybrid pollen)	85.3 (non-sprayed hybrid leaf)
PAT	<LOQ (pollen, forage and grain)	11.2 (non-sprayed hybrid).

*ng/mg tissue dry weight

[1] The hybrid DAS-59122-7 line consisted of backcross 1 (BC_1) generation seed, produced from crossing the DAS-59122-7 T_0 plants twice with a recurrent inbred line and then with a different inbred line.

[2] The inbred DAS-59122-7 line consisted of BC_1 generation seed, produced from backcrossing the 59122-7 T_0 plants twice with a recurrent inbred

Table 5: Summary of expression levels of Cry34Ab1 protein in DAS-59122-7 corn grain harvested at maturity

	Mean (ng/mg dry weight)	Standard deviation	Range (ng/mg dry weight) [1]	Number of samples[2]
Non-GM control	0	0	0-0	6/6
GM hybrid unsprayed	49.7	16.2	28.9-84.8	30/0
GM hybrid sprayed	61.1	19.4	30.9-117	30/0
GM inbred	51.7	11.5	38.6-78.2	15/0

[1]The limit of quantitation (LOQ) for Cry34Ab1 for grain was 0.072 ng/mg dry weight.
[2]Number of samples = the number of samples analysed/the number of samples below the LOQ

Table 6: Summary of expression levels of Cry35Ab1 protein in DAS-59122-7 corn grain harvested at maturity

	Mean (ng/mg dry weight)	Standard deviation	Range (ng/mg dry weight) [1]	Number of samples[2]
Non-GM control	0	0	0-0	6/6
GM hybrid unsprayed	0.99	0.33	0.48-1.58	30/0
GM hybrid sprayed	0.92	0.30	0.50-1.61	30/0
GM inbred	1.10	0.54	0-1.83	15/2

[1]The limit of quantitation (LOQ) for Cry35Ab1 for grain was 0.072 ng/mg dry weight.
[2]Number of samples = the number of samples analysed/the number of samples below the LOQ

Table 7: Summary of expression levels of PAT protein in DAS-59122-7 corn grain harvested at maturity

	Mean (ng/mg dry weight)	Standard deviation	Range (ng/mg dry weight) [1]	Number of samples[2]
Non-GM control	0	0	0-0	6/6
GM hybrid unsprayed	0	0	0-0	30/30
GM hybrid sprayed	0	0	0-0	30/30
GM inbred	0	0	0-0	15/15

[1]The limit of quantitation (LOQ) for PAT for grain was 0.06 ng/mg dry weight.
[2]Number of samples = the number of samples analysed/the number of samples below the LOQ

Potential dietary exposure to novel proteins

The highest level of expression of Cry34Ab1 and Cry35Ab1 in the grain of DAS-59122-7 corn based on the expression data above was 117 ng/mg and 1.83 ng/mg dry weight respectively. The actual exposure to these two proteins in the diet is expected to be lower than this due to a number of factors including:

- protein degradation during the transport and storage of grain,
- grain containing these novel proteins is likely to be mixed with other non-GM and GM corn grain and thus dilute the novel proteins, and
- reductions in the protein concentrations during processing to produce high fructose corn syrup and vegetable oils (which contain negligible levels of protein).

Even at the highest levels of novel protein expression, and without accounting for the above factors, which are expected to lower the dietary exposure, the levels are extremely low i.e. 12 mg Cry34Ab1/100g corn and 0.2 mg Cry35Ab1/100g corn.

Potential toxicity of novel proteins

Proteins which cause toxicity act via acute mechanisms and generally at very low doses (Sjoblad *et al.*, 1992). Therefore, when a protein demonstrates no acute oral toxicity at a high dose level using a standard laboratory mammalian test species, this supports the determination that the protein will be non-toxic to humans and other mammals, and will not present a hazard under any realistic exposure scenario, including long term exposures.

Studies submitted:
Schafer, BW, Collins, RA, Schwedler DA, and Xu X (2003) Characterisation of Cry34Ab1 and Cry35Ab1 Proteins derived from transgenic maize event E4497.59.1.22 (DAS-59122-7). Dow AgroSciences LLC, Indianapolis Indiana. Study ID: 030033

Korjagin, V.A. (2000) Characterisation of *Pseudomonas* produced and transgenic maize expressed phosphinothricin acetyltransferase (PAT) protein. Global Environmental Chemistry Laboratory – Indianapolis Lab. Dow AgroSciences LLC, Indiana. Study ID: 000369.

Brooks, K.J. and DeWildt, P.M. (2000) PS149B1 14 kDa Protein: Acute Oral Toxicity Study in Cd-1 Mice. Toxicology and Environmental Research and Consulting, The Dow Chemical Company. Study ID 001130

Brooks, K.J. and DeWildt, P.M. (2000) PS149B1 44 kDa Protein: Acute Oral Toxicity Study in CD-1 Mice. Toxicology and Environmental Research and Consulting, The Dow Chemical Company. Study ID 001129

Brooks, K.J. and DeWildt, P.M. (2000) PS149B1 14 kDa and 44 kDa Proteins: Acute Oral Toxicity Study in CD-1 mice. Toxicology and Environmental Research and Consulting, The Dow Chemical Company. Study ID 001128

Brooks, K.J. (2000) PAT Microbial Protein (FL): Acute Oral Toxicity Study in CD-1 Mice. Toxicology and Environmental Research and Consulting, The Dow Chemical Company. Study ID:991249

Three acute oral toxicity studies in mice where assessed to support the safety of the Cry34Ab1 and Cry35Ab1 proteins: Cry34Ab1 only; Cry35Ab1 only; and a mixture of both Cry34Ab1 and Cry35Ab1.

As it is very difficult to extract and purify sufficient quantities of the subject protein from transgenic corn plants for the acute oral toxicity studies, it has become standard practice to instead use equivalent proteins that have been produced using bacterial expression systems. Prior to use, the bacterially produced proteins are compared to the proteins produced *in planta* in order to establish their equivalence. Cry34Ab1 and Cry35Ab1 proteins were produced in recombinant *Pseudomonas fluorescens*.

The molecular identity and biochemical characteristics of the proteins expressed *in planta* and in the bacterial-expression systems were examined using various biochemical methods such as N-terminal sequencing, molecular weight determination, immunoreactivity, glycosylation analysis, peptide mass fingerprinting and matrix assisted laser desorption ionisation time of flight (MALDI-TOF) mass spectrometry. These studies established that bacterially produced Cry proteins were equivalent to those proteins produced in corn line DAS-59122-7, thus the bacterial proteins were used in the toxicity testing.

Potential toxicity of Cry34Ab1 and Cry35Ab1 individually

The acute oral toxicity of the two Cry proteins, both individually and combined was studied using an acute oral toxicity study in mice. The Cry proteins were produced in *Pseudomonas fluorescens*.

Test material	PS149B1 14 kDa protein (54% Cry34Ab1) or PS149B1 44 kDa protein (37% Cry35Ab1)
Vehicle	0.5% aqueous methylcellulose
Test Species	5 male CD-1 mice for each of two test materials
Dose	5000 mg/kg body weight (2700 mg Cry34Ab1/kg body weight, or 1850 mg Cry35Ab1/kg body weight) in one gavage dose of 25 mL/kg
Control	No control was performed

The mice received a single dose of either 2700 mg/kg bw Cry34Ab1 or 1850 mg/kg bw Cry35Ab1 and were observed for two weeks. Parameters evaluated included body weights and detailed clinical observations. All animals were observed for gross pathological changes.

All mice survived the two-week observation period. No clinical signs were observed during the study. Three mice given Cry34Ab1 and two mice given Cry35Ab1 lost weight between days 1 and 2 but gained weight for the rest of the study period. One mouse given Cry35Ab1 had fluctuating body weight throughout the study. This was thought to be due to gavage with a maximum volume of methylcellulose. The remaining mice gained weight throughout the study. There were no treatment related gross pathological observations.

Therefore, under the conditions of this study, the acute oral LD_{50} of Cry34Ab1 in male mice is greater than 2700 mg/kg bw and of Cry35Ab1 is greater than 1850 mg/kg bw.

Potential toxicity of Cry34Ab1 and Cry35Ab1 combined

As Cry34Ab1 and Cry35Ab1 are present together in corn line DAS-59122-7 and are required to be expressed together to be effective in combating corn rootworm, an acute oral toxicity study in mice using a combination of the two novel proteins was performed.

Test material	a mixture of PS149B1 14 kDa protein and 44 kDa protein (at a 1:3 ratio of Cry34Ab1 to Cry35Ab1 to provide an equimolar mixture of the two proteins)
Vehicle	0.5% aqueous methylcellulose
Test Species	5 male and 5 female CD-1 mice
Dose	5000 mg/kg body weight (482 mg Cry34Ab1/kg bw and 1520 mg Cry35Ab1/kg bw) in one gavage dose of 25 mL/kg
Control	No control was performed

The mice received a single dose of 482 mg/kg bw Cry34Ab1 and 1520 mg/kg bw Cry35Ab1 and were observed for two weeks. Parameters evaluated included body weights and detailed clinical observations. All animals were observed for gross pathological changes.

All mice survived the two-week observation period. One female mouse had protruding or enlarged eyes on test days 6 and 7, however this was not considered to be treatment related. No other clinical signs were observed during the study. Two male mice lost weight between days 1 and 2 but gained weight over the rest of the study period. The remaining mice gained weight throughout the study. There were no treatment related gross pathological observations.

Therefore, under the conditions of this study, the acute oral LD_{50} of a 1:3 mixture of Cry34Ab1 and Cry35Ab1 in CD-1 mice is greater than 2000 mg /kg bw (482 mg Cry34Ab1 /kg bw and 1520 mg Cry35Ab1 /kg bw).

Potential toxicity of PAT

Extensive animal testing has shown that the PAT protein is non-toxic to humans and animals. The same gene has been expressed in other transgenic crops assessed by FSANZ (applications A372, A375, A386, A481, and A518) and is considered to pose no risks to human health and safety.

However, an acute oral toxicity study of the PAT protein in 5 male and 5 female CD-1 mice was also assessed.

Test material	PAT protein produced in *Pseudomonas florescenes* (84% pure)
Vehicle	0.5% aqueous methylcellulose
Test Species	5 male and 5 female CD-1 mice
Dose	6000 mg/kg body weight (5000 mg/kg PAT) in two gavage doses of one hour apart
Control	No control was performed

Parameters evaluated included body weights and detailed clinical observations. All animals were observed for gross pathological changes.

All mice survived the two-week observation period. One female mouse had increased pupil size on test days –1 to 6, however this was not considered to be treatment related. No other clinical signs were observed during the study.

All mice had a decrease in body weight between days 1 and 2. This was minor, transient and typical of high volume gavage doses, and not attributed to the test material. All mice except one female gained weight over the rest of the study. One female lost 0.5 gm over the duration of the study. There were no gross pathological lesions on any animal in the study.

Therefore, under the conditions of this study, the acute oral LD_{50} of the PAT protein in CD-1 mice is greater than 5000 mg /kg bw.

Potential toxicity of glufosinate ammonium metabolites

Glufosinate ammonium herbicide contains both the L-isomer and the D-isomer of glufosinate. Unlike the L-isomer, the D-isomer does not competitively inhibit the glutamine synthase enzyme in plants and is not herbicidally active. In plants expressing the *pat* gene, the herbicidally active component of glufosinate ammonium, the L-isomer, is rapidly metabolized by the action of the enzyme phosphinothricin acetyltransferase (PAT) into the non-phytotoxic stable metabolite N-acetyl-L-glufosinate (2-acetamido-4-methylphosphinico-butanoic acid) (NAG). This metabolite does not inhibit glutamine synthetase, therefore the plants will survive applications of this herbicide (OECD, 2002a).

The toxicity of NAG and a second metabolite of glufosinate ammonium produced by both non-tolerant and tolerant plants, 3-[hydroxy(methyl) phosphinoyl]propionic acid was compared with that of glufosinate ammonium by the Joint meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group (JMPR) in 1999. JMPR concluded that the toxicity of the metabolites was comparable to or less than that of the parent compound. An Acceptable Daily Intake (ADI) was established for this group of 0-0.02 mg/kg bw for glufosinate ammonium, NAG and 3-[hydroxy(methyl) phosphinoyl]propionic acid (alone or in combination). Due to the low acute toxicity of glufosinate-ammonium and its metabolites, it was considered unnecessary to establish an acute reference dose (JMPR, 1999).

Similarities with known protein toxins

Studies Submitted:
Cressman, R.F. (2003) Evaluation of the Sequence Similarities of the Cry34Ab1, Cry35Ab1, and PAT Proteins to the Public Protein Sequence Datasets. Pioneer Hi-Bred International Inc. Study ID: PHI-2003-046

Bioinformatic analyses assessed the Cry34Ab1, Cry35Ab1 and PAT proteins for any similarity with known protein toxins. The similarity search was conducted against the GenPept dataset using the BLASTP 2.2.6 algorithm with a cut-off expectation (E) value of

1.0.

Cry34Ab1

The Cry34Ab1 similarity search identified ten proteins. Five of these represent closely related or identical Cry proteins from *B. thuringiensis*. The other five represent putative microbial collagenases and hypothetical proteins from several genome sequencing projects. None of the similar proteins were identified as toxins or potential toxins.

Cry35Ab1

The results of the Cry35Ab1 protein search returned 22 protein accessions with E-values of less than 1. Seven of these were highly similar or identical Cry proteins from *B. thuringiensis*. Eleven were from a related species, *B. sphaericus*. Four represent conceptual or hypothetical proteins from genome sequencing projects. None were identified as toxins or potential toxins.

PAT

Searching the dataset with the PAT protein revealed 148 accessions, 18 of which represent accessions for PAT or other acetyltransferases. The remaining 130 proteins are unidentified proteins and / or hypothetical proteins translated from genome sequencing data. Again, none of the similar proteins returned by the search were identified as toxins or potential toxins.

Conclusion

The data from acute oral toxicity studies and bioinformatic analyses of the novel proteins indicate that none of the three proteins are toxic at high levels in mice, nor do they show any similarity with known protein toxins.

Potential allergenicity of novel proteins

A possible concern is that new proteins introduced into food will cause allergic reactions in some individuals. The potential allergenicity of a novel protein is evaluated using an integrated, step-wise, case-by-case approach relying on various criteria used in combination, since no single criterion is sufficiently predictive of either allergenicity or non-allergenicity. The assessment focuses on the source of the novel protein, any significant amino acid similarity between the novel protein and that of known allergens, and the structural properties of the novel protein, including susceptibility to degradation in simulated digestion models. Applying such criteria systematically provides reasonable evidence about the potential of the newly introduced proteins to act as an allergen (Jones and Maryanski, 1991; Lehrer and Reese, 1998).

The two Cry proteins expressed in corn line DAS-59122-7 were assessed using these criteria for their potential allergenicity.

Similarity to known allergens

Studies submitted:
Song, P. (2003) Comparison of the Amino Acid Sequence of *Bacillus thuringiensis* Strain PS149B1 Cry34Ab1 and Cry35Ab1 Insecticidal Crystal Proteins as Expressed in Maize to Known Protein Allergens. Dow AgroSciences LLC, Indiana. Study ID: GH-C 5671

Stelman, S.J. (2000) Comparison of the Amino Acid Sequence of *Bacillus thuringiensis* Strain PS149B1 13.6 kDa and 43.8 kDa Insecticidal Crystal Proteins to Known Protein Allergens. Dow AgroSciences LLC, San Diego, California. Study ID: GH-C 5140

A comparison on the amino acid sequence of the introduced proteins to known protein allergens is one of the steps in a multilevel decision tree to assess allergenic potential (Metcalfe *et al.*, 1996).

Sequence evaluation guidelines based on those formulated by Gendel (1998), by the Joint FAO/WHO Expert Consultation (2001) and by the Codex Alimentarius Commission (2001) were followed (Gendel, 1998; Joint FAO/WHO expert consultation on allergenicity of foods derived from biotechnology, 2001; Codex, 2001; Joint FAO/WHO expert consultation, 2001). An immunologically significant sequence identity requires a match of at least eight contiguous identical amino acids, or 35% identity over eighty amino acid residues. No such sequence identity was detected for either the Cry34Ab1 or Cry35Ab1 sequences. Therefore, based on homology of the amino acid sequences with known protein allergens, the Cry34Ab1 and Cry35Ab1 sequences are not predicted to have allergenic potential.

In vitro digestibility

Studies submitted
Korjagin,V.A. and Ernest, A.D. (2000) *In vitro* Digestibility of PS149B1 Proteins. Global Environmental Chemistry Laboratory – Indianapolis Lab. Dow AgroSciences LLC, Indiana. Study ID: 000302

Herman, R.A., Schafer, B.W., Korjagin, V.A. and Ernest, A.D. (2003) Rapid Digestion of Cry34Ab1 and Cry35Ab1 in Simulated Gastric Fluid. *J. Agric. Food Chem*. 51:6823-6827.

Typically, most food allergens tend to be stable to the peptic and acidic conditions of the digestive system if they are to reach and pass through the intestinal mucosa to elicit an allergic response (Astwood and Fuchs, 1996; Metcalfe *et al.,* 1996; Kimber *et al*., 1999). The Cry1Ac and Cry1F proteins were therefore investigated for their digestibility in simulated digestion models.

Two studies showing the *in vitro* digestibility of the two Cry proteins were performed. In the first study (Korjagin and Ernest, 2000), Cry34Ab1 was degraded in simulated gastric fluid (SGF) after 30 minutes incubation at 37ºC, as determined by Western blotting. Cry35Ab1 was degraded more quickly, with no visible hybridisation after just 5 minutes at 37ºC.

In the second study (Herman *et al*., 2003) a more quantitative approach was taken. More than 97% of the Cry35Ab1 was found to be degraded after 5 minutes incubation with SGF at 37ºC, based on a limit of detection of the SDS-PAGE of <15.6 ng/lane. In two

experiments, the estimated half-life of Cry34Ab1 in SGF at 30ºC was 1.9 and 2.0 minutes. The time taken for 90% of the sample to be degraded under the same conditions was 6.3 and 6.8 minutes based on SDS-PAGE analysis. This is comparable to other *Bt* proteins that have been used in GM plants (Herman *et al*., 2003).

Thermolability

Studies submitted:
Herman, R.A. (2000) Thermolability of PS149B1 Binary Delta-Endotoxin. Global Environmental Chemistry Laboratory – Indianapolis Lab. Dow AgroSciences LLC Indiana. Study ID: 001041

Herman, R.A. (2002) Heat Lability of Individual Proteins of the PS149B1 Binary ICP. Global Regulatory Laboratories – Indianapolis Lab, Dow AgroSciences LLC Indiana. Study ID: 010144

Two studies assessing the thermolability of the Cry34Ab1 and Cry35Ab1 proteins were examined.

A mixture of both Cry proteins (at a ratio of 1:1) were incubated for 30 minutes at 4ºC (control), 60ºC, 75ºC and 90ºC. These samples were then fed to Southern corn rootworm (SCR) neonate larvae as part of their standard feed. The variable measured was growth inhibition of SCR larvae. This is a qualitative assessment of heat lability as the rate of denaturation is not directly obtainable since a reduction in biological activity cannot be directly linked to protein concentration. Also, other factors, such as the properties of the buffer and concentration of the heated samples will affect the rate of denaturation. Therefore these studies can only give a qualitative statement of “heat stable” or “heat labile”.

After 6 days on the treated diet, the weight of the larvae was measured and the growth inhibition was calculated based on comparison with negative controls. The results of this study indicated that the protein mixture was deactivated after exposure to 60ºC, 75ºC and 90ºC for 30 minutes.

Treatment		% Growth Inhibition*
Cry34/35Ab1	4ºC	70
Cry34/35Ab1 60ºC		-3
Cry34/35Ab1 75ºC		-3
Cry34/35Ab1 90ºC		1

* As the Cry34/35Ab1 protein complex is toxic to the larvae, measuring the % growth inhibition in larvae fed diets containing this complex gives an indication of the functionality of the complex after treatment at various temperatures. At 4ºC the protein is functional and causes 70% growth inhibition in treated larvae compared to the control larvae. Following heat treatment (at 60ºC, 75ºC or 90ºC) the protein complex is no longer functional and does not cause any growth inhibition in larvae compared to control larvae.

A second study was conducted to determine the heat lability of the individual component proteins by fortifying heated samples of the two proteins with non-heated samples of the individual proteins. This allowed the heat lability of the complementary protein to be measured since both proteins are required for maximum activity against corn rootworm. This study showed that the Cry35Ab1 protein is heat labile at 60ºC, 75ºC, and 90ºC. The Cry34Ab1 protein was also found to be heat labile at 90ºC, however, some Cry34Ab1 activity was observed at 60ºC and 75ºC.

Conclusion regarding characterisation of the novel proteins

Corn line DAS-59122-7 expresses three novel proteins – Cry34Ab1, Cry35Ab1, expressed at low levels in the corn grain, and PAT which is undetectable in the corn grain.

A number of studies have been done on these proteins to determine their potential toxicity and allergenicity. These studies demonstrate that the proteins are non-toxic to mammals, and have limited potential to be allergenic.

COMPARATIVE ANALYSES

Most crops, including oilseed crops, exhibit considerable variability in their nutrient composition. Environmental factors and the genotype of the plant have an enormous impact on composition. Thus, variation in these nutrient parameters is a natural phenomenon and is considered to be normal.

A comparative approach focussing on the determination of similarities and differences between the GM food and its conventional counterpart aids in the identification of potential safety and nutritional issues and is considered the most appropriate strategy for the safety and nutritional assessment of GM foods (WHO, 2000). The critical components to be measured are determined by identifying key nutrients, key toxicants and anti-nutrients for the food source in question (FAO, 1996).

The key nutrients and toxicants/anti-nutrients are those components in a particular food that may have a substantial impact in the overall diet. These may be major constituents (e.g., fats, proteins, carbohydrates) or minor components (e.g., minerals, vitamins). Key toxicants are those toxicologically significant compounds known to be inherently present in the plant, such as those compounds whose toxic potency and level may be significant to health (e.g., solanine in potatoes if the level is increased). The key components of corn that should be considered in the comparison include protein, fat, carbohydrates, amino acids, fatty acids, vitamins, minerals, and phytic acid (OECD, 2002b).

Nutrient analysis

Study submitted
Essner R. (2003) Agronomic characteristics, quantitative ELISA and nutrient composition analysis of hybrid maize lines containing Cry34Ab1, Cry35Ab1 and PAT genes: Chile locations. Pioneer Hi-bred International, Inc. Study ID: PHI-2002-050

To determine whether unexpected changes had occurred in the nutrient composition of corn line DAS-59122-7 as a result of the genetic modification, and to assess the nutritional adequacy of this line, compositional analysis was done on whole corn grain from corn line DAS-59122-7 and from its non-transgenic counterpart. The non-transgenic counterpart used as a control was a near isoline corn, which has the same genetic background as corn line DAS-59122-7 without the insert.

Corn line DAS-59122-7 and the control corn line were grown at 6 different locations in 2002-2003. Plots of the transgenic corn were either left untreated or received two sequential applications of a herbicide containing the active ingredient glufosinate ammonium. Five grain samples (single ears of corn) were collected from each treatment group at each location. One sample was collected from the control group at each location.

A total of 51 components were analysed - these were proximate content (moisture, fat, protein, fibre, ash and carbohydrate), amino acids, fatty acids, minerals, vitamins, secondary metabolites, and antinutrients.

The results were compared within and across sites. Comparisons across all locations are shown in Tables 8-13 and discussed below to evaluate the overall equivalence of DAS-59122-7 corn grain with conventional corn. The results from individual trial sites were also evaluated but are not presented in this report.

Of the 102 comparisons across sites, 34 comparisons were found to be significantly different at the 5% level. Every single one of these differences, however, was within the literature range and represented only a small difference compared to the control value. Furthermore, there was no pattern of change within sites that might indicate that further investigation is necessary.

Beta-carotene levels in the GM corn grain (sprayed and unsprayed) were higher than reported averages, but were comparable to the control mean, which was also higher than the literature range. This may be due to other xanthophylls or carotenoid pigments inadvertently being measured as beta-carotene. Levels of vitamin B2 were below the limit of quantitation for the assay used for this analysis and were not detected.

These minor differences are unlikely to be biologically meaningful, and the grain and forage from DAS-59122-7 corn can be considered to be compositionally equivalent to that of non-GM corn.

Conclusion of compositional analysis

The comparative analyses do not indicate that there are any compositional differences of biological significance in corn grain from transgenic corn line DAS-59122-7, compared to the non-GM control. Several minor differences in key nutrients and other constituents were noted, however, the levels observed were generally within the range of natural variation for commercial corn lines and do not indicate an overall pattern of change that would warrant further investigation. On the whole, it can be concluded that DAS-59122-7 corn grain is equivalent in composition to non-GM corn grain.

Table 8: Summary of proximate and fibre analysis in DAS-59122-7 corn grain (across sites)

Analyte[1]	Literature range[2]	Mean[3]		
		DAS-59122-7 unsprayed	DAS-59122-7 sprayed	Control
Crude protein	6-16.1	10.0*	10.3*	9.61
Crude fat	1.2-18.8	4.69	4.62	4.49
Crude fibre	1.6-5.5	2.3	2.2	2.3
ADF[4]	1.82-11.3	3.5	3.6	3.5
NDF[5]	3.0-22.6	10.8	11.2*	10.3
Ash	0.62-6.28	1.55*	1.6*	1.42
Carbohydrate[6]	63.3-89.8	83.8	83.5*	84.5

[1]Per cent dry weight
[2]Watson, 1982 and 1987; Jugenheimer, 1976; OECD, 2002; ILSI, 2003; Essner, 2003
[3]Least square means
[4]Acid detergent fibre
[5]Neutral detergent fibre
[6]Carbohydrates are calculated using the following formula = 100% - % protein - % fat - % ash
*Statistically significant difference between DAS-59122-7 grain and control grain ($P<0.05$).

Table 9: Summary of mineral analysis of DAS-59122-7 corn grain (across sites)

Analyte[1]	Literature range[2]	Mean[3]		
		DAS-59122-7 unsprayed	DAS-59122-7 sprayed	Control
Calcium	0.002-0.1	0.00278*	0.00286*	0.00227
Phosphorus	0.21-0.75	0.299	0.308*	0.266
Copper	0.000085-0.001	0.000112	0.000104	0.000118
Iron	0.0001-0.01	0.00199	0.00225	0.00194
Magnesium	0.08-1.0	0.117	0.123	0.108
Manganese	0.00007-0.0054	0.000648	0.000686*	0.000577
Potassium	0.28-0.72	0.352	0.362	0.332
Sodium	0.0-0.15	0.000437	0.000367	0.000378
Zinc	0.00065-0.0037	0.00183	0.00179	0.00163

[1]Per cent dry weight
[2]Watson, 1982 and 1987; OECD, 2002; ILSI, 2003.
[3]Least square means
*Statistically significant difference between DAS-59122-7 grain and control grain ($P<0.05$).

Table 10: Summary of fatty acid analysis of DAS-59122-7 corn grain (across sites)

Analyte[1]	Literature range[2]	Mean[3]		
		DAS-59122-7 unsprayed	DAS-59122-7 sprayed	Control
Palmitic acid	6.51-19	11.5*	11.7	12.1
Stearic acid	0-4.17	1.39*	1.40*	1.57
Oleic acid	18.6-46	22.8	23.1	23.3
Linoleic acid	34-70	63.0*	62.4	61.7
Linolenic acid	0-2.0	1.14	1.15*	1.07

[1]Percent total fatty acids
[2]Watson, 1982; Iowa Gold Catalog, 1997; Essner, 2003; ILSI, 2003.
[3]Least square means
*Statistically significant difference between DAS-59122-7 grain and control grain ($P<0.05$).

Table 11: Summary of amino acid analysis in DAS-59122-7 corn grain (across sites)

Analyte[1]	Literature range[2]	Mean[3]		
		DAS-59122-7 unsprayed	DAS-59122-7 sprayed	Control
Methionine	0.1-0.46	0.20	0.19	0.19
Cysteine	0.08-0.32	0.23	0.22	0.22
Lysine	0.05-0.55	0.28	0.29	0.28
Tryptophan	0.04-0.13	0.06*	0.06	0.06
Threonine	0.21-0.58	0.38	0.41*	0.37
Isoleucine	0.19-0.71	0.34*	0.35*	0.33
Histidine	0.15-0.40	0.26*	0.28*	0.25
Valine	0.21-0.85	0.46*	0.48*	0.45
Leucine	0.43-2.41	1.33*	1.38*	1.28
Arginine	0.22-0.64	0.29*	0.30*	0.28
Phenylalanine	0.04-0.83	0.56*	0.59*	0.54
Glycine	0.24-0.50	0.35	0.36*	0.33
Alanine	0.37-1.20	0.82	0.83*	0.80
Aspartic acid	0.37-0.95	0.69	0.70*	0.66
Glutamic acid	0.89-3.04	2.03	2.08*	1.97
Proline	0.43-1.46	0.96*	0.98*	0.91
Serine	0.24-0.91	0.51	0.54*	0.50
Tyrosine	0.11-0.79	0.24*	0.26*	0.21

[1]Per cent dry weight
[2]Watson, 1982; Iowa Gold Catalog, 1994, 1997; OECD, 2002; Essner, 2003; ILSI, 2003; Pioneer Commercial Hybrids.
[3]Least square means
*Statistically significant difference between DAS-59122-7 grain and control grain (P<0.05).

Table 12: Summary of vitamin analysis of DAS-59122-7 corn grain (across sites)

Analyte[1]	Literature range[2]	Mean[3]		
		DAS-59122-7 unsprayed	DAS-59122-7 sprayed	Control
Beta-carotene	1.0, 2.5[4]	7.62	7.74	6.87
Vitamin B1	1.0-8.6	5.45	5.93	5.77
Vitamin B2	0.25-16.5	ND	ND	ND
Folic acid	0.147 – 1.209[5]	0.593*	0.603	0.634
Vitamin E[6]	1.5-6.87	6.59*	6.60*	5.65

[1]parts per million on a dry weight basis
[2]Watson, 1982, 1987; OECD 2002; ILSI version 1 2003.
[3]Least square means
[4]ILSI version 1 – 1 ppm, OECD – 2.5 ppm average
[5] ILSI version 2 2004.
[6]Measured as α-tocopherol
ND – not detected
*Statistically significant difference between DAS-59122-7 grain and control grain (P<0.05).

Table 13: Summary of secondary metabolites and anti-nutrients of DAS-59122-7 corn grain (across sites)

Analyte[1]	Literature range[2]	Mean[3]		
		DAS-59122-7 unsprayed	DAS-59122-7 sprayed	Control
Secondary metabolites				
Inositol	NR	0.022	0.022	0.021
Raffinose	0.08-0.31	0.13	0.13	0.12
Furfural	NR	ND	ND	ND
P-Coumaric acid	0.003-0.058	0.014	0.014	0.015
Ferulic acid	0.02-0.37	0.177	0.176	0.182
Antinutrients				
Phytic acid	0.29-1.29	0.877	0.798	0.798
Trypsin inhibitor (TIU/g)	1.1-7.18	2.82	2.84	2.84

[1]Per cent dry weight
[2]Watson, 1982; OECD, 2002; ILSI, 2003.
[3]Least square means
NR – Not reported
ND – Not detected
*Statistically significant difference between DAS-59122-7 grain and control grain ($P<0.05$).

NUTRITIONAL IMPACT

Studies submitted
Malley, L.A. (2004) Thirteen-week feeding study with transgenic maize grain (DAS-59122-7) in rats. Unpublished Pioneer Hi-Bred International sponsored study. Study ID Du-Pont-13910

Smith, B. (2003) Nutritional Equivalency Study of Maize Containing Cry34Ab1 and Cry35Ab1: Poultry Feeding Study. Solution BioSciences Inc. Study ID: 2001-OPT-48-BB

In assessing the safety and suitability of a GM food, a key factor is the need to establish that the food is nutritionally adequate and will support typical growth and wellbeing. 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.

To date, all approved GM plants with modified agronomic production traits have been shown to be compositionally equivalent to their conventional counterparts. Feeding studies with feeds derived from the approved GM plants have shown equivalent animal performance to that observed with the non-GM feed. Thus the evidence to date is that for GM varieties shown to be compositionally equivalent to conventional varieties, feeding studies with target livestock species will add little to a safety assessment and generally are not warranted.

For plants engineered with the intention of significantly changing their composition/nutrient bioavailability and thus their nutritional characteristics, however, suitable comparators may not be available for a nutritional assessment based solely on compositional analysis. In such cases feeding trials with one or more target species may be useful to demonstrate wholesomeness for the animal.

In the case of corn line DAS-59122-7, the extent of the compositional and other available data is considered to be adequate to establish the nutritional adequacy of the food. However, a 3-month feeding study with DAS-59122-7 corn grain in rats was also assessed by FSANZ.

It is important to note that the study, while based on the protocol for a sub-chronic toxicity study, is a comparative feeding study with different varieties of corn. As such, its overall usefulness in assessing the safety of DAS-59122-7 corn or its constituents is limited because of the limits on the amount of test material that can be incorporated in an animal's diet without creating a nutritional imbalance. In this particular study, the highest level of incorporation in the diet of DAS-59122-7 corn was 35%. The ability of feeding studies to detect adverse effects from a constituent present in a food product will be largely dependent on the intrinsic toxicity of any such constituent and whether it is present in the food in a sufficient amount to induce toxicity under the conditions of the study. Notwithstanding these limitations, and providing the study has been well designed and executed, the absence of any adverse effects may however provide additional assurances of safety.

In this study, groups of young adult male and female Crl:CD®(SD)IGS BR rats (12/sex/group) were administered a diet containing 35% DAS-59122-7 corn grain incorporated into a traditional rodent diet (Rodent Chow 5002). For comparison, four additional groups of rats were fed diets produced with a near isoline non-transgenic hybrid maize line (091), non-transgenic commercial hybrid maize line (33R77), or one of two separate lots of commercially available rodent chow (designated 5002A and 5002B). Rats were fed the diet for approximately 90 days. Body weights, food consumption and clinical signs were evaluated weekly. Neurobehavioural and ophthalmologic assessments were performed prior to the start of dietary exposure and near the end of the exposure period. Clinical pathology end points were evaluated at approximately 45 days and 90 days. After approximately 90 days of dietary exposure (days 92 – 93 for males; days 93 – 94 for females), rats were killed and gross and microscopic pathological examinations were conducted on all animals used in the study. The following tissues were collected from all rats and examined: liver, oesophagus, stomach, duodenum, jejunum, ileum, caecum, colon, rectum, salivary glands, pancreas, kidneys, bladder, lungs, trachea, nose, larynx/pharynx, heart, aorta, spleen, thymus, mandibular and mesenteric lymph nodes, bone marrow, pituitary, thyroid, parathyroid, adrenals, brain, spinal cord, sciatic nerve, skeletal muscle, femur/knee joint, sternum, testes, epididymides, prostate, seminal vesicles, ovaries, uterus, mammary glands, vagina, skin, eyes and gross lesions.

There were no test related effects on body weight or body weight gain over the course of the study. A statistically significant increase in body weight gain was observed in males consuming the diet containing DAS-59122-7 corn for days 77-84, however this was transient and was not considered to be related to consumption of corn line DAS-59122-7. Body weight gain in DAS-59122-7 corn fed males was low during test days 42-49 due to one animal that lost weight. The weight loss was due to the feeder ring top jamming the feed jar and preventing one rat from accessing his feed.

Food consumption and food efficiency were similar between groups for both males and females. A statistically significant increase in mean food efficiency was observed in males fed corn line DAS-59122-7 during test days 1-2, 35-42 and 77-84, however these differences were not considered to be related to the test diet as increased food efficiency was not consistently observed over the study.

No treatment related deaths occurred. One female rat in the reference group (5002B) was killed *in extremis* on test day 70, but the death was not attributed to food consumption. No test-related abnormal clinical signs were observed. One male rat in the DAS-59112-7 fed group showed focal retinal degeneration, a common spontaneous lesion in rats of this ages, which was not considered to be test related.

There were some statistically significant differences in the haematology of rats fed diets containing DAS-59122-7 grain compared with the other groups. Mean corpuscular haemoglobin concentration (MCHC) was increased in males fed DAS-59122-7 corn grain at test days 44 and 92 (by 1% and 3%, respectively) compared to the combined control groups (fed 5002A, 5002B, 091 or 33R77) and at day 92 compared to the individual control groups. However the average MCHC was within the historical range and therefore not

considered to be test diet related. Reticulocyte counts were significantly lower (12%) in males at test day 92 compared to the combined control groups, however there were no differences when the test group was compared with individual control groups. The mean reticulocyte counts of the group of test males, the 5002B fed group and the 33R77 fed group were all lower than the historical range, but as other red cell mass parameters (red cell counts, haemoglobin concentration and haematocrit) were normal, the decreased reticulocyte counts were considered to be chance finding and unrelated to consumption of DAS-59122-7 grain.
Red cell distribution width (RDW) was statistically significantly decreased (4%) in males at day 92 compared to the combined control groups, but not the individual control groups. This minimal decrease was not considered to be test related. RDW was significantly decreased (3%) in females fed the DAS-59122-7 diet at day 45, but not at day 92, when compared to the combined control groups. Compared to the individual control groups a significant difference was only observed compared to the 091 group. Mean RDW for females were within the historical range and therefore this minor decrease was not considered to be biologically significant. Platelet counts were significantly decreased in males and increased in females fed the diet containing DAS-59122-7 grain when compared to the combined control groups at study termination (12% and 14% respectively). When platelet counts were compared to individual control groups, the only significant difference was for females consuming DAS-59122-7 compared to those consuming 5002A. These differences were not considered by the study author to be related to the test diet. Absolute monocytes counts were significantly decreased (33%) at test day 93 in females fed the DAS-59122-7 diet compared to the combined control groups. When compared to the individual control groups, the only significant difference was with rats fed the 5002A diet. This was within the historical range and not considered to be biologically significant. There was a significant increase (5%) in activated partial thromboplastin time (APTT) in males after 92 days when compared to the combined control groups. No significant difference was observed when compared with individual control groups and the mean APTT was within the historical range and therefore not considered to be test related.

There were some statistically significant differences in the clinical chemistry of rats fed the DAS-59122-7 grain diet, however these differences were not considered to be biologically significant. Total protein was increased (4%) at test days 93-94 in females compared to the combined control groups, no differences were observed when compared with the individual control groups however. The increased total protein resulted from significantly increased albumin concentrations (6%), this was within the historical range and therefore not considered to be test related.

Calcium and potassium concentrations were significantly decreased (by 2% mad 4% respectively) at day 44 in males fed DAS-59122-7 diets compared to the combined control groups, however the only difference when compared to the individual control groups was with the group fed diet 5002A. These changes were small and transient and therefore the pathologist did not consider them to be biologically significant. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were increased (although not statistically significantly) on day 45 in females fed DAS-59122-7 grain by 17% and 70% respectively,

compared to the combined control groups. This was due to one female rat with extremely high levels of these two enzymes. At day 93, this rat had AST and ALT levels comparable to others in the group and the mean values for the group were similar to the other control groups.

Urine volume was statistically significantly decreased (43%) in males fed DAS-59122-7 grain at day 92 when compared to the combined control groups. No difference was found when compared to the individual control groups. A non-statistically significant increase (26%) in osmolality in males after 92 days compared to the combined control groups was also found, but not considered to be treatment related by the study authors.

A statistically significant increase in uterine weight (both absolute and relative to body and brain weights) was observed in females fed DAS-59122-7 grain diets compared to the combined control groups. However, rat uterine weights vary based on the stage of oestrus, with peak weight occurring during the stages of proestrus and oestrus. When compared to the individual control groups, uterine weights were significantly increased compared to females in the 5002A, 091 and 33R77 groups), but not compared to the 5002B group. The 5002B group had a similar number of females in either proestrus or oestrus to the DAS-59122-7 group (7 out of 12 compared to 8 out of 11), whereas the other groups had fewer animals in these stages (5002A and 091 each had 2 out of 12, 33R77 had 5 out of 12). Therefore the study authors considered the increase in uterine weight to be due to variation in oestrus cycle rather than the DAS-59122-7 diet.

Under the conditions of this study, consumption of DAS-59122-7 corn grain by male and female rats at a level of 35% in the diet produced no adverse effects and was comparable to commercially available rodent chow and non-GM corn varieties.

The results of a 42-day feeding study of a similar GM corn (containing the *cry34Ab1*, *cry35Ab1*, and *pat* genes) in commercial broiler chickens showed no adverse effects.

REFERENCES

An, G., Mitra, A., Choi, H.K., Costa, M.A., An, K., Thornburg, R.W. and Ryan, C.A. (1989) Functional analysis of the 3' control region of the potato wound-inducible proteinase inhibitor II gene. *Plant Cell* 1(1):115-122.

Armstrong, C.L., Green, C.E. and Phillips, R.L. (1991) Development and availability of germplasm with high type II culture formation response. *Maize Genetics Co-operation Newsletter* 65:92-93.

Astwood, J.D. and Fuchs, R.L. (1996) Allergenicity of foods derived from transgenic plants. *Highlights in food allergy. Monographs in Allergy*, 32. 105-120.

Baum, J.A., Chu, C.R., Rupar, M., Brown, G.R., Donovan, W.P., Huesing, J.E., Ilagan, O., Malvar, T.M., Pleau, M., Walters, M. and Vaughn, T. (2004) Binary toxins from Bacillus thuringiensis active against the western corn rootworm, Diabrotica virgifera virgifera LeConte. *Appl Environ Microbiol* 70(8):4889-4898.

Betz, F.S., Hammond, B.G. and Fuchs, R.L. (2000) Safety and Advantages of Bacillus thuringiensis-Protected Plants to Control Insect Pests. *Regulatory Toxicology and Pharmacology* 32:156-173.

CFIA (1994) *The Biology of Zea mays L. (Corn/Maize)*. Canadian Food Inspection Agency, Ottawa.

Christensen, A.H., Sharrock, R.A. and Quail, P.H. (1992) Maize polyubiquitin genes: structure, thermal perturbation of expression and transcript splicing, and promoter activity following transfer to protoplasts by electroporation. *Plant Mol Biol* 18(4):675-689.

Codex (2001) *Codex Ad hoc Intergovernmental Task Force on Foods Derived from Biotechnology, Report of the Working Group on Allergenicity.*, Vancouver, Canada.

EPA (1998) *RED Facts: Bacillus thuringiensis*. EPA 738-F-98-001., Environmental Protection Agency.

FAO. (1996) *Biotechnology and food safety*. A report of a Joint FAO/WHO Consultation. *Food and Agriculture Organisation, Food and Nutrition Paper 61, Food and Agriculture Organization of the United Nations, Rome.*

FAO. (2001) FAOSTAT Database; Provisional 2001 production and production indices data.

Gendel, S.M. (1998) The use of amino acid sequence alignments to assess potential allergenicity of proteins used in genetically modified foods. *Adv.Food Nutr Res* 42:45-62.

Hertig, C., Rebmann, G., Bull, J., Mauch, F. and Dudler, R. (1991) Sequence and tissue-specific expression of a putative peroxidase gene from wheat (Triticum aestivum L.). *Plant Mol Biol* 16(1):171-174.

Hofte, H. and Whiteley, H.R. (1989) Insecticidal crystal proteins of Bacillus thuringiensis. *Microbiol Rev* 53(2):242-255.

Hohn, T., Richards, K. and Genevieve, L. (1982) Cauliflower mosaic virus on its way to becoming a useful plant vector. *Curr.Top.Microbiol Immunol.* 96:194-236.

IPCS. (1999) *Bacillus thuringiensis*. Environmental Health Criteria of the International Programme on Chemical Safety, No 217. http://www.who.int/pcs/ehc/summaries/ehc_217.html. Accessed on

JMPR (1999) *Pesticide Residues in Food: Toxicological Evaluations, Glufosinate Ammonium*. http://www.inchem.org/documents/jmpr/jmpmono/v99pr06.htm.

Joint FAO/WHO expert consultation (2001) *Joint FAO/WHO expert consultation on allergenicity of foods derived from biotechnology, Evaluation of allergenicity of genetically modified foods*.

Joint FAO/WHO expert consultation on allergenicity of foods derived from biotechnology (2001) *Evaluation of allergenicity of genetically modified foods*.

Jones, D.D. and Maryanski, J.H. (1991) Safety considerations in the evaluation of transgenic plants for human food. In: Levin, M.A. and Strauss, H.S. eds. *Risk assessment in genetic engineering*. McGraw-Hill, New York,

Kimber, I., Kerkvliet, N.I., Taylor, S.L., Astwood, J.D., Sarlo, K. and Dearman, R.J. (1999) Toxicology of protein allergenicity: prediction and characterization. *Toxicol.Sci* 48(2):157-162.

Lehrer, S.B. and Reese, G. (1998) Food allergens: implications for biotechnology. In: Thomas, J.A. eds. *Biotechnology and safety assessment*. Taylor and Francis, Philadelphia,

Masson, L., Schwab, G., Mazza, A., Brousseau, R., Potvin, L. and Schwartz, J.L. (2004) A novel Bacillus thuringiensis (PS149B1) containing a Cry34Ab1/Cry35Ab1 binary toxin specific for the western corn rootworm Diabrotica virgifera virgifera LeConte forms ion channels in lipid membranes. *Biochemistry* 43(38):12349-12357.

McClintock, J.T., Schaffer, C.R. and Sjoblad, R.D. (1995) A comparative review of the mammalian toxicity of *Bacillus thuringiensis*-based pesticides. *Pestic.Sci.* 45:95-105.

Metcalfe, D.D., Astwood, J.D., Townsend, R., Sampson, H.A., Taylor, S.L. and Fuchs, R.L. (1996) Assessment of the allergenic potential of foods derived from genetically engineered crop plants. *Crit Rev Food Sci Nutr* 36 Suppl:S165-S186.

OECD (2002a) *Series on Harmonsitation of Regulatory Oversight in Biotechnology, No. 25. Module II: Phosphinothricin*. Organisation for Economic Co-operation and Development.

OECD (2002b) *Series on the Safety of Novel Foods and Feeds, No 6. Consensus Document on Compositional Considerations for New Varieties of Maize (Zea mays): Key Food and Feed Nutrients, Anti-nutrients and Secondary Plant Metabolites*. Organisation for Economic Cooperation and Development, Paris.

Schnepf, E., Crickmore, N., Van Rie, J., Lereclus, D., Baum, J., Feitelson, J., Zeigler, D.R. and Dean, D.H. (1998) Bacillus thuringiensis and its pesticidal crystal proteins. *Microbiol Mol Biol Rev* 62(3):775-806.

Sjoblad, R.D., McClintock, J.T. and Engler, R. (1992) Toxicological considerations for protein components of biological pesticide products. *Regul.Toxicol.Pharmacol.* 15(1):3-9.

Tinland B. and Hohn B. (1995) Recombination between prokaryotic and eukaryotic DNA: integration of Agrobacterium tumefaciens T-DNA into the plant genome. *Genet Eng* 17:209-229.

USDA (1995) *Availability of determination of non regulated status for genetically engineered corn*. United States Department of Agriculture, Washington.

White, P.J. and Pollak, L.M. (1995) Corn as a food source in the United States: Part II. Processes, products, composition, and nutritive values. *Cereal Foods World 40*. pp756-762.

WHO. (2000) Safety aspects of genetically modified foods of plant origin. Report of a Joint FAO/WHO Expert Consultation, World Health Organization, Geneva.