FOOD DERIVED FROM GLUFOSINATE AMMONIUM TOLERANT CORN LINE T25

A Safety Assessment

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SUMMARY

Food derived from glufosinate ammonium-tolerant corn line T25 has been assessed to determine its suitability for human consumption. The evaluation criteria included characterisation of the transferred genes, analysis of changes at the DNA, protein and whole food levels, stability of the introduced genes, evaluation of intended and unintended changes and assessment of the potential allergenicity or toxicity of any newly expressed proteins.

Nature of the genetic modification

Herbicide tolerant corn line T25 has been developed to provide growers with a crop that is tolerant to applications of the broad spectrum herbicide, glufosinate ammonium. This trait has been introduced into corn line T25 by addition of the *pat* gene, which encodes phosphinothricin acetyl transferase, the PAT protein. PAT confers tolerance to glufosinate ammonium herbicide by acetylating phosphinothricin, the active component of the herbicide.

A truncated ampicillin resistance gene (*bla*) is also present in corn line T25. This gene is non-functional in the plant.

The transferred novel genetic material is stably integrated at a single site and maintained in the corn over multiple generations. The novel genetic material in corn line T25 comprises only a minute fraction of the total DNA present in the corn and is therefore unlikely to pose any additional risks.

History of use

Corn has been cultivated for centuries and is used as a basic food item by people throughout the world. It is a staple food for a significant proportion of the world's population. Corn-based products are routinely used in a wide range of foods and have a long history of safe use. Sweet corn varieties are grown for human consumption. Grain and by-products from processing of corn are also used as animal feedstuffs.

Characterisation of novel protein

Only one new protein is expressed in corn line T25: phosphinothricin acetyltransferase (PAT). This protein, encoded by the *pat* gene, allows plants to detoxify the broad-spectrum herbicide phosphinothricin (the active moiety of glufosinate ammonium herbicide). The PAT protein has a very narrow substrate specificity for phosphinothricin and demethyl-phosphinothricin, neither of which are found in humans. Acetyl transferases are a class of enzymes common to all bacterial, plant and animal cells and play a major role in both the synthesis and oxidation of fats. Although the PAT protein is not normally consumed, *Streptomyces*, from which it is derived, is a common soil bacterium, which may be found on and around plant produce.

The *pat* gene is expressed at low levels in corn line T25. PAT protein is translated at the highest levels in the plant silage tissue and is much lower in the kernel where it represents less than 0.0005% total protein. The level of DNA and protein in highly processed corn based products are expected to be very low and in some cases, negligible. It is also likely that the proteins will be degraded and/or removed during processing steps.

The PAT protein loses enzymatic activity immediately upon exposure to gastric pH, and is readily digested in the stomach. The PAT protein does not show any similarity with known toxins or allergens.

Comparative analyses

Detailed compositional analyses were undertaken to establish the nutritional adequacy of corn line T25, and to compare it to non-modified control lines. No consistent differences in corn components or nutrients were observed in genetically modified corn line T25 compared to controls, or in glufosinate ammonium -treated plants compared to untreated controls. Although some statistically significant differences were observed, these were small and did not have any biological significance or raise any safety concerns. All reported values fell within the range cited in the published literature.

An animal feeding study using chickens provided additional supporting data for the safety of corn line T25. The results of this study confirm the results of the compositional studies and demonstrate that corn line T25 is able to support typical growth and well-being.

Overall, these results demonstrate that corn line T25 is compositionally similar to non-modified corn hybrids, and support the conclusion that unintended secondary effects are unlikely to have occurred as a result of the genetic modification.

Conclusion

No potential public health and safety concerns have been identified in the assessment of glufosinate ammonium tolerant corn line T25. On the basis of the data provided in the present application, herbicide-tolerant corn line T25 is equivalent to other commercially available corn in terms of its safety and nutritional adequacy.

FOOD DERIVED FROM GLUFOSINATE AMMONIUM TOLERANT CORN LINE T25:

A SAFETY ASSESSMENT

BACKGROUND

A safety assessment has been conducted on food derived from corn that has been genetically modified to be tolerant to the herbicide glufosinate ammonium. The modified corn is referred to as glufosinate ammonium tolerant corn line T25.

Glufosinate ammonium (also referred to as phosphinothricin) is a non-selective, contact herbicide that provides effective post-emergence control of many broadleaf and grassy weeds. The mode of action of the herbicide is to inhibit the enzyme glutamine synthetase, an essential enzyme involved with ammonium accumulation and nitrogen metabolism is plants. The inhibition of glutamine synthetase results in an over accumulation of ammonia in the plant, which leads to cell death. Tolerance to glufosinate ammonium is conferred though the expression in the plant of the enzyme phosphinothricin acetyl transferase (PAT), encoded by the *pat* gene from the soil bacteria *Streptomyces viridochromogenes*. The production of PAT by corn line T25 enables the use of glufosinate ammonium herbicides post-emergence without risk of damaging the crop. The development of GM glufosinate ammonium tolerant corn will provide a selective use for glufosinate ammonium, creating a valuable new weed management tool for corn producers.

Corn products derived from glufosinate ammonium tolerant corn line T25 are most likely to be imported as processed food products.

HISTORY OF USE

Corn (*Zea mays* L., also called *maize*) has a long history of safe use as a food for both humans and other animals. Being the only important cereal crop indigenous to North America, it has been utilised for thousands of years and was the foundation of the extensive North and South American ancient civilisations. Corn seed was carried to Europe centuries ago, where it became established as an important crop in southern latitudes, moving rapidly to Africa, Asia and other parts of the world.

In countries where corn is an important crop, it is the principal component of livestock feeds, and most of it is fed to farm animals, particularly to ruminants. In only a few countries is corn a major constituent of human diets. In developed countries, corn is consumed mainly as popcorn, sweet corn, corn snack foods and occasionally as corn bread. However, many 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.

In the United States, corn is the largest crop in terms of planted acreage, total production and crop value (National Corn Growers Association, 1997). While corn is generally used as a high energy animal feed, it is also a very suitable raw material for the manufacture of starch which is largely converted to a variety of products for human consumption, such as sweetener and fermentation products including high fructose corn syrup and ethanol. Corn oil is commercially processed from the germ and accounts for approximately nine percent of domestic vegetable oil production. Little whole kernel or processed corn is consumed by humans worldwide when compared to these corn-based food ingredients that are used in the manufacture of many foods including bakery and dairy goods, beverages, confections and meat products.

DESCRIPTION OF THE GENETIC MODIFICATION

Methods used in the genetic modification

Corn line T25 was developed from a parental tissue culture line, He/89, which was transformed with a plasmid containing the *pat* gene and the *bla* gene. The *pat* gene confers glufosinate ammonium tolerance and the *bla* gene confers resistance to the antibiotic ampicillin. The *bla* gene has been truncated during transformation and is non-functional in the plant. The transformed plant cells were produced by direct DNA uptake by protoplast cultures with additional DNA. The full details of the method of transformation and the proprietary plasmid used in the transformation have been provided to Food Standards Australia New Zealand (FSANZ) for assessment, however, at the request of the applicant, this information has been accepted as confidential commercial information under the FSANZ Act 1991.

Function and regulation of novel genes

The *pat* gene is derived from the soil micro-organism *Streptomyces viridochromogenes* strain Tu494. It codes for the enzyme phosphinothricin acetyl transferase (PAT), which modifies and inactivates the herbicide glufosinate ammonium (Strauch *et al*, 1988).

The *pat* gene is often used as a selectable marker to distinguish genetically modified plant cells from unmodified cells. In this application, the *pat* gene has been transferred to corn to confer tolerance to glufosinate ammonium herbicides. The *pat* gene is under the control of the cauliflower mosaic virus (35S CaMV) promoter (Odell *et al*, 1985) and termination signal (Pietrzak *et al*, 1986). The CaMV 35S promoter drives constitutive expression of the *pat* gene throughout the plant. The bacterial *pat* gene contains a high G:C content that is not typical of plant genes. To optimise expression in plants, a synthetic gene that has a lower G:C content has been transferred to corn and has approximately 70% DNA sequence similarity with the native gene. However, the amino acid sequence of the PAT protein has not been altered (Wohlleben *et al*, 1988; Strauch *et al*, 1988).

In addition to the *pat* gene, T25 corn was transformed with a partial *bla* gene. The *bla* gene is derived from *Eschericia coli* and encodes β-lactamase, which confers resistance to some β-lactam antibiotics, including penicillin and ampicillin. The *bla* gene is under the control of bacterial regulatory sequences and was used as a selectable marker to distinguish transformed bacterial cells from non-transformed cells. The transformed plant contains a truncated form of the *bla* gene, i.e. it is missing 25% of its 5' sequence. This shortened form of the gene is not functional in the modified corn.

Characterisation of the genes in the plant

Southern blot experiments of the original T25 transformant and a third generation of back-crossing with line B73, confirmed the presence of the *pat* gene and a truncated *bla* gene in the genetically modified corn line. The transformation process resulted in a single copy of the *pat* and *bla* genes being integrated into T25 corn, as confirmed by full sequenced analysis of the DNA insert. During the transformation of the corn cells, the *bla* gene was disrupted (25% of the gene sequence was truncated) and is consequently not functional in the plant. This is supported by polymerase chain reaction (PCR) analysis and also that no β–lactamase activity was detected by HPLC-radio-monitoring as discussed below in Protein Expression Analysis.

Stability of genetic changes

Studies evaluated:
Klonus D (1998). Genomic characterization of maize transformant T25. Performing laboratory: Hoescht Schering Aventis GmbH, Department D-65926, Frankfurt am Main, Federal Republic of Germany. Report No. PSR98/029.

Southern analysis was performed to determine the stability of the transgenic locus over multiple generations. Two generations of corn were tested; the original transformant and the third generation of back-crossing with the parental line B73. The inbred line B73 and non-transgenic regenerate plants of line HE89 were used as controls. The DNA fragments hybridising with a *pat* probe on Southern blots were identical in the original transformant and subsequent back-crossed generations, indicating stability of the DNA introduced into the corn.

Plants screened for phenotypic stability, i.e. tolerance to the herbicide glufosinate ammonium, demonstrated inheritance patterns consistent with a single insertion site that was stable over multiple generations.

Impact on human health from the potential transfer of novel genetic material to cells of the human digestive tract

The potential human health impact of transfer of novel genetic material to cells of the human digestive tract depends on the nature of the novel genes and must be assessed on a case-by-case basis.

In 1991, the World Health Organization (WHO) issued a report of a Joint FAO[1]/WHO Consultation , which looked at strategies for assessing the safety of foods produced by biotechnology (WHO 1991). The consultation concluded that as DNA from all living organisms is structurally similar, the presence of transferred DNA in food products, in itself, poses no health risk to consumers.

The major concern in relation to the transfer of novel genetic material to gut micro-organisms is in regard to 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 micro-organisms present in the human digestive tract and that this could compromise the therapeutic use of antibiotics. However, no functional antibiotic resistance gene was transferred to corn line T25, as indicated by a range of analyses including PCR, enzyme assay and northern analysis. Therefore, as the disrupted *bla* gene is not functional and there is no functional gene product, it is not considered to pose any safety risk.

In relation to the transfer of other novel genetic material from GM food to bacteria in the human digestive system, this is extremely unlikely to occur because of the number of complex and unlikely steps that would need to take place consecutively.

It is equally unlikely that novel genetic material from genetically modified food would transfer to human cells via the digestive tract. In considering the potential impact on human health, 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.

[1] Food and Agriculture Organization.

Conclusion

A single copy of the *pat* and truncated *bla* genes are transferred to corn resulting in the development of herbicide tolerant (glufosinate ammonium) corn line T25. Segregation analyses indicate that the transferred DNA is integrated into the corn genome as a single and stable insert.

CHARACTERISATION OF NOVEL PROTEIN

Biochemical function and phenotypic effects

PAT

Only one new protein is expressed in corn line T25: phosphinothricin acetyltransferase (PAT). This protein, encoded by the *pat* gene, allows plants to detoxify the broad-spectrum herbicide phosphinothricin (the active moiety of glufosinate ammonium herbicide). The PAT protein has a very narrow substrate specificity for phosphinothricin and demethyl-phosphinothricin, both of which are not found in humans. Acetyl transferases are a class of enzymes common to all bacterial, plant and animal cells and play a major role in both the synthesis and oxidation of fats. Although the PAT protein is not normally consumed, *Streptomyces* is a common soil bacterium, which can be found on and around plant produce.

In plants, the enzyme glutamine synthetase plays a central role in the uptake of nitrogen by catalysing the incorporation of ammonia into glutamine. The herbicide glufosinate ammonium inhibits this enzyme in plants, leading to an accumulation of ammonia in the tissues, which kills the plant. The PAT protein catalyses the acetylation of phosphinothricin, thus eliminating its herbicidal activity (Strauch *et al*, 1988). Acetylation of phosphinothricin produces N-acetyl-glufosinate (NAG) and two further metabolites, 3-methylphosphinico-propionic acid (MPP) and 3-methylphosphinicoacetic acid (MPA).

The level of expression of the PAT protein in corn line T25 is sufficiently high for the corn to be marketed as herbicide tolerant. No additional substrates, apart from phosphinothricin, have been reported.

Protein expression analyses

The level of expression and activity of the PAT protein has been determined, in order to establish potential dietary exposure to this protein. Studies to confirm that the *bla* gene is non-functional were also done.

Studies evaluated:

Klonus D. 1999. Expression of the phosphinothricin acetyltransferase in glufosinate tolerant T25 corn. Hoechst Schering Aventis GmbH, Germany. Report PSR99/005

Klonus D. 1999. Polymerase chain reaction analysis (PCR) of the glufosinate tolerant maize line T25.

Schulz 1993. Beta-lactamase activity in Ignite (Glufosinate ammonium based herbicide) resistant corn

Van Wert S and Forster V (1996). Composition, nutrition and/or amount of phosphinothricin acetyltransferase in whole and processed fractions of glufosinate resistant corn: transformation events T14 and T25 and nontransgenic counterparts. Performing laboratory: Aventis USA Company, Wilmington DE, United States. Report No. BK-95B-01.

PAT protein

PAT protein levels were analysed in several plant tissues including the mature kernel, forage (tasseling to silking), silage (late milk to early dough) and fodder (dry plant at harvest) collected from two field locations (Indiana and Puerto Rico) during the 1994 growing season. Isogenic or near isogenic parental corn lines (i.e. same genetic makeup except for the novel gene) were used as controls.

A double-antibody enzyme-linked immunosorbent assay (ELISA) was used to quantify the PAT protein from T25 hybrids and their isogenic controls. The ELISA detects both intact and denatured PAT, therefore the results are likely to overestimate the level of active protein. The limit of detection of the assay is approximately 1 ng PAT/mg protein.

No PAT protein was detected in any control plants or in kernels of T25 hybrid plants grown in Indiana (Table 1). Grain from inbred T25 corn grown in Puerto Rico contained 4.02 ng PAT/mg protein (± 0.62 ng/mg protein), representing a maximum of 0.00046% of the crude protein in the kernel. The highest level of protein was found in the silage, containing 119.24 ng PAT/mg protein representing a maximum of 0.00132% total protein. It is significant that most commercial corn lines are hybrids rather than inbred lines and these results indicate that PAT protein levels are virtually non-detectable in hybrid lines.

Table 1: Level of PAT protein in corn tissues from hybrid and inbred T25 corn[1]

		Mean levels ± standard deviation (ng/mg protein)[2]			
Indiana		**kernel**	**silage**	**forage**	**fodder**
Hybrid T25	T25-1	nd	14.82 ± 0.86	-	-
	T25-2	nd	12.51 ± 1.38	-	-
	T25-2[3]	nd	14.81 ± 1.30	-	-
Control	*T25*	nd	nd	-	-
Puerto Rico					
Inbred T25	T25-3	4.02 ± 0.62	119.24 ± 13.36	62.70 ± 40.07	79.91 ± 5.23
Control	*T25*	nd	nd	nd	nd

[1]Values are expressed as ng PAT/mg protein. At least three samples were analysed in duplicate.
[2]nd = not detected; '-' = analysis not done. Limit of detection is approximately 1 ng PAT/mg protein.
[3]Plants were treated with glufosinate ammonium at the V8 stage (ie 69 days prior to harvest of silage, 115 days prior to harvest of fodder and grain).

PAT activity

PAT activity was measured in kernels, leaves, roots, stem and pollen of greenhouse grown flowering T25 corn plants. The enzyme was detectable in kernels, leaves, roots and stems but not in pollen. The highest activity was found in stems (62.54 µMol N-acetyl-glufosinate produced/minute/mg protein extract). The activity in kernels was significantly lower (less than 60 times lower), ranging from 0.192 - 1.29 µMol N-acetyl-glufosinate produced/ minute/ mg protein extract.

In most processed corn products, i.e. those produced from dry- and wet-milling corn fractions (including flakes and grits), it is unlikely that the enzyme is active, given that the corn is processed using temperatures up to 105°C (220°F) and this is sufficiently high to denature proteins and thus inactivate the enzyme.

Equivalence of the plant PAT protein to the bacterially produced protein

It was shown by SDS-PAGE that the PAT protein produced in corn line T25 has a molecular mass of approximately 22 kiloDaltons. The kinetics and substrate specificity of the protein were characterised and PAT was found to be highly specific for the substrate L-phosphinothricin, with a very low affinity

for related compounds and amino acids. High temperatures and extremes of pH were found to inactivate PAT. These properties of the PAT protein extracted from T25 corn were found to be indistinguishable from the protein extracted from *E. coli.*

β-lactamase

The glufosinate ammonium tolerant corn line T25 contains a single truncated copy of the bacterial *bla* gene. The gene was disrupted during transformation of the corn cells and is missing 25% of the gene sequence. Northern analyses were used to verify that the *bla* gene is neither transcribed to produce a stable transcript nor translated into an active protein with a β-lactamase function. β-lactamase activity in the genetically modified corn was assessed by HPLC-radio-monitoring. The standard assay contained 4.25 pmol of radio-labelled penicillin and β-lactamase activity could be detected in 1:500 dilutions of a bacterial culture fluid with a protein concentration as low as 6.6 μg/ml. No β-lactamase activity was detected in protein extracts of young growing leaves from wildtype and transgenic corn plants, at protein concentrations of 500–1000 fold higher than and incubation times of up to 12 times longer than the bacterial system. It can be concluded that the partial *bla* gene from the plasmid vector does not produce an active β-lactamase in the herbicide-tolerant corn line T25.

Potential toxicity of novel protein

Studies evaluated:

Kuhn JO (1995). Phosphinothricin acetyltransferase (Sample PAT-0195) Acute oral toxicity study in mice. Performing Laboratory: Stillmeadow Inc, Sugar Land, Texas. Sponsor: Ciba-Geigy Corporation, Research Triangle Park, NC

Kuhn JO (1995). Phosphinothricin acetyltransferase (Sample PAT-0195) Acute oral toxicity study in mice. Performing Laboratory: Stillmeadow Inc, Sugar Land, Texas. Sponsor: Ciba-Geigy Corporation, Research Triangle Park, NC

The only new protein present in glufosinate ammonium tolerant corn line T25 is the PAT protein. The acute oral toxicity of the PAT protein has been evaluated by FSANZ previously in regard to glufosinate ammonium tolerant and insect protected corn lines Bt-176 and Bt-11 and glufosinate ammonium tolerant DBT-418 corn. The following studies on the acute toxicity and physical and chemical characteristics of the PAT protein have been previously assessed.

Similarity with known toxins

A comparison of the amino acid sequence of the PAT protein to the sequences of known toxins present in public databases (EMBL, Swissprot), demonstrated that it does not share any significant similarity with any known protein toxins. Additionally, no reports were found of toxicity associated with acetyl transferases as a class and the donor organism has no known pathogenic potential.

Acute oral toxicity in mice – bacterially produced PAT

To further show that PAT is non-toxic, an acute toxicity test in mice was conducted. The scientific basis for using an acute test is that known protein toxins generally act via acute mechanisms (Jones and Maryanski 1991). Hsd:S-D ICR albino mice (source: Harlan Sprague Dawley Inc, Texas) were housed individually in controlled conditions with free access to food and water, except for the 16 hours before dosing when food was withheld. Groups (5/sex) of mice were given a single oral dose (gavage) of PAT protein (PAT-0195, purity 51% phosphinothricin acetyltransferase, expressed by the *bar* gene in *E. coli*) in carboxymethyl cellulose; heat inactivated PAT (PAT-0195C, 52% purity) in carboxymethyl cellulose; or carboxymethyl cellulose to a total dose of PAT protein of approximately 2600 mg/kg bw (ie 51-52% of 5050 mg/kg bw, given that this was the purity of the protein).

Mice were observed for clinical signs at least 3 times on the day of dosing and once daily after this for a 14-day observation period. Bodyweight was determined predosing (day 0) and on days 7 and 14. At the end of the study, mice were killed for postmortem examination of gross pathology.

One male receiving the test substance died during the study. The only notable clinical signs were decreased activity, piloerection and ptosis (drooping eyelid) on days 6–8 in the male that died. One male receiving the reference substance showed slight piloerection on the day of dosing. However, as no other clinical signs were observed in animals of any group, these signs are not considered to be treatment related. Bodyweight gain was unaffected by treatment, except in the male that died. There were no abnormal findings on postmortem of animals surviving until the end of the study. The results do not indicate any potential toxicity from the PAT protein.

Potential allergenicity of novel protein

Allergic reactions to foods are relatively rare and are generally associated with a small group of well characterised proteins found in common foods such as milk from dairy cows, wheat, soybeans, fish and tree nuts. For the vast majority of the population, consumption of these foods is without adverse effects.

However, there is some concern that novel proteins introduced into food may elicit an allergic response. Although there are no simple predictive assays available to assess the allergic potential of proteins, a number of characteristics are common among many of the allergens that have been characterised. For instance, amino acid sequence similarity with known allergens may be a useful gauge of allergenic potential. A string of 8-12 consecutive amino acid residues in common with known allergens could be an indicator for allergenicity given that many T-cell epitopes of allergenic proteins are that length (Taylor and Lehrer, 1996). In terms of the chemical and physical nature of proteins, known allergens tend to be glycosylated proteins with a molecular weight of 10–70 KDa (Lehrer *et al*, 1996). Allergens also tend to be heat stable as well as resistant to peptic and tryptic digestion and the acidic conditions of the stomach. Consequently, many allergenic factors tend to be resistant to proteolytic digestion (Taylor and Lehrer, 1996). The PAT protein was evaluated for potential allergenicity against these criteria: amino acid sequence similarity to known allergens, and how easily the protein is degraded by heat, acid and gastric enzymes (Lehrer and Reese 1998, Jones and Maryanski 1991).

Studies evaluated:

Privalle L (1994). *In vitro* digestibility and inactivation of the *bar* marker gene product phosphinothricin acetyltransferase (PAT) under simulated mammalian gastric conditions. Ciba Seeds. Agricultural Biotechnology Research Unit, Ciba-Geigy Corporation, Research Triangle Park, NC, USA.

Schneider, R., 1993. Fate of introduced DNA in gut: Degradation of phosphinotrhicin acetyl transferase gene from transgenic rape HCN 92 (Brassica napus) in stomach fluids from pig, chicken and cow. Hoechst AG Agricultural Division, Frankfurt am Main, Germany. Study No. BR 93/06

Schulz A (1993) L-phosphinothricin N-acetyltransferase, biochemical chacterization. Performing laboratory: Biologische Forschung C, Biochemie der Pflanzen, Hoechst Aktiengessellschaft, Frankfurt. Report No. 93.01.

Van Wert S and V Forster. 1996. Composition, nutrition and/or amount of phosphinothricin acetyl transferase in whole and processed fractions of glufosinate resistant corn: Transformation events T14 and T25 and non-transgenic counterparts. Performing Laboratory: Xenos, Woodson-Tenent, Texas A&M, Agrevo.

Comparison of PAT with known allergens

No similarity was found between the sequence of the PAT protein and sequences of known allergens in a search of public databases. Additionally, acetyltransferases in general have no similarity to any reported mammalian allergens.

Effect of simulated gastric conditions on activity of the PAT protein

The PAT protein was assessed for stability in simulated gastric juices. Enzyme samples were incubated at 37°C in stomach fluid from beagle dogs, for up to 15 minutes. Over the course of the incubation, samples were removed, diluted in buffer, adjusted to pH 8 and assayed immediately for enzyme activity. The original pH of the stomach fluid was 1.1, experiments were repeated with stomach fluid adjusted to pH 4, to determine the effect that pH modulating medications (taken for stomach disorders) might have on digestion of the protein.

Purified PAT in stomach fluid of pH 1.1 was rapidly inactivated, losing all activity within one minute. At pH 4, inactivation was slower, taking up to 10 minutes. Crude PAT was again inactivated rapidly by gastric juice at pH 1.1, at the higher pH inactivation of the crude protein was slower than that of the purified enzyme. This study supports the conclusion that any PAT protein consumed in foods derived from corn line T25 will be readily denatured upon entering an acidic environment, such as the human gut.

Digestibility of PAT protein

As potential allergenicity is often related to the presence of large, undigested protein molecules, it can be useful to look at the digestibility of a novel protein.

The PAT protein used in this trial was obtained from an *E. coli* expression system and was purified following fermentation. Simulated gastric fluid (SGF) contained NaCl (2 mg/mL), HCl and pepsin (3.2 mg/mL), the pH was 1.0 to 1.2, and the activity of the fluid was determined before use. Samples were taken at zero and two minutes. The presence of PAT in the fluid following incubation was determined by SDS-PAGE analysis. PAT enzymic activity was also determined at the pH optimum for the enzyme, at gastric pH and following serial incubation with a gastric solution containing 0.0032 mg/mL pepsin.

In the presence of SGF containing a standard concentration of pepsin, the PAT protein was completely degraded at time zero. After 2 minutes of incubation with 0.1 or 0.01 times the standard pepsin concentration, PAT degradation appeared complete. When 0.001 times the standard pepsin concentration was used, a significant amount of PAT remained after a 2-minute incubation period. This concentration was thus selected for the enzyme inactivation studies.

The enzyme activity of PAT decreased to 56% of initial values after a 10-minute incubation at 37^{o}C. This reflects the thermal sensitivity of the enzyme above 35^{o}C, and would represent the maximum activity were gastric pH or pepsin to have no effect on PAT activity. Immediately after addition to SGF without pepsin, PAT activity decreased to 2.6% of the initial activity, and reached zero by 1 minute. When pepsin was included in the SGF, the initial activity was even lower. Activity was not restored by neutralisation, indicating that inactivation of the PAT enzyme was irreversible. The half-life of the PAT protein in SGF containing 0.0032 mg/mL was between 1 and 2 minutes.

This study demonstrates that the PAT protein is readily digested in the gastric environments and is not likely to be allergenic.

Conclusion

Although the PAT protein would not normally be present in the food supply, it is sourced from a common soil bacterium and is not considered to pose a health and safety risk. Additionally, the class of enzymes PAT belongs to is very commonly found in all bacterial, plant and animal cells and has a major metabolic role.

The PAT protein is expressed at low levels in corn line T25. It is expressed at the highest levels in the plant silage tissue and is much lower in the kernel where it represents less than 0.0005% total protein. The level of DNA and protein in highly processed corn based products are expected to be very low and in some cases, negligible. It is also likely that the proteins will be degraded and/or removed during processing steps.

PAT protein loses enzymatic activity immediately upon exposure to gastric pH, and that the protein is readily digested in the stomach. The PAT protein does not show any similarity with known allergens. Therefore it is highly unlikely that the PAT protein would be toxic or allergenic to humans.

COMPARATIVE ANALYSES

The safety assessment of foods produced using gene technology entails, in this case, evaluating compositional data from the transgenic corn plant in comparison with equivalent data from the parental (or non-transformed) plant line or literature values for the particular crop species. This process involves identifying the key components, including nutrients and any toxicants, characteristic of corn grain and also takes into account the variation in composition due to genetic variability, environmental factors, and post-harvest handling and processing.

Studies evaluated:

Van Wert S (1999). Composition and nutrition of corn grain: a comparison of transgenic hybrids T25-2 and T25-5 in Canada and in the USA. Performing laboratory: Woodson-Tenent Laboratories Inc, Technical Assessment Systems Inc. Report No. C003219.

Van Wert S (1996). Composition of whole fractions of glufosinate resistant corn untreated and treated with glufosinate: Transformation event T25. Performing laboratory: AgrEvo USA Company, Willmington DE, United States. Report No. A55781.

Nutrient analysis

The overall nutritional composition of corn line T25, including any changes resulting from the genetic modification, has been assessed. The composition of the food derived from corn line T25 has been compared with other commercial varieties of the crop. The major components examined were protein, moisture, ash, fibre, fat, carbohydrates, calcium, phosphorous, fatty acids and amino acid composition. Where there are statistically significant differences between the genetically modified and the conventionally bred crop, further comparisons can be made with values available in the literature to determine whether the parameter is within the normal range for non-transformed lines. As T25 is marketed as a herbicide-tolerant variety, the impact of glufosinate ammonium application on the biochemical composition of kernels has been assessed.

The composition of two hybrids derived from transformation event T25 (T25-2 and T25-5) was assessed. Plants were grown in two sets of field trials: the first field trial was conducted over two consecutive years in 1994–95 in the USA (hybrid T25-2) and the second field trial was conducted at two sites (Breslau and Ridgetown, Ontario) in Canada in 1995 (hybrid T25-5). The genetically modified hybrid T25-2 was developed by backcrossing the original transformant to another inbred line. T25-2 was backcrossed to this inbred line three times in 1994 and four times in 1995. The genetically modified hybrid T25-5 was developed by backcrossing the original transformant four times to another inbred line. These hybrids are considered to have 87.5% and 93.25% genetic homology, respectively. The genetically modified lines and their non-genetically modified counterparts are not true isolines but are considered genetic counterparts.

At each location, genetically modified plants and their respective non-genetically modified counterparts were planted in a randomised block design. Plants were harvested at maturity and at least three representative samples from each site, for each genetic background, were analysed.

Analysis included moisture, fat, protein, fibre, ash, carbohydrate (by calculation), fatty acid composition, amino acid composition and minerals.

The samples of genetically modified and non-genetically modified corn were collected and nutrient analyses data was analysed statistically. The data was analysed using multiple comparisons (i.e. between sites or years, within country and combined countries) to determine any significant differences between the genetically modified and non-genetically modified counterparts.

In separate analyses of the data from each of the USA and the Canadian field trials, there were some significant differences between genetically modified and control corn lines. The values for all components are listed in Tables 2 (USA data) and Table 3 (Canadian data). The United States Department of Agriculture's Handbook 8 values for these components have also been listed as a reference.

USA

Significant differences in the proximate parameters protein and carbohydrate, in the fatty acids C18:3 and C20:0, in the minerals calcium and phosphorus and in seven amino acids (Table 2) were observed between the genetically modified T25-2 and control corn line. Additional statistical analyses of the data, separating data according to year, determined that there was an effect of season on many of these nutrient parameters. The data was collected over two years, with different environmental conditions each year (temperature, rainfall, soil moisture and type). Additionally, these differences between the genetically modified and non-genetically modified corn were not consistent between years. Although these differences were identified as statistically significant, they are all within the range given in the United States Department of Agriculture's Handbook 8.

Canada

T25-5 corn grown in Canada showed significant differences in the fatty acids C18:1, C18:2 and C20:0, and in the amino acid cystine (Table 3). C18:2 was at a higher level in the transgenic than in the isogenic corn, whereas C18:1 and C20:0 were lower in the transgenic samples. Additional statistical analyses of the data, separating data according to sites, determined that there was an effect of location on some of these nutrient parameters. The samples were collected from two sites, with different environmental conditions at each site (temperature, rainfall, soil moisture and type), which could account for the location effect.

The lack of consistent differences between the wild-type hybrids and their modified counterparts suggests that these effects are likely to be due to normal variation, rather than an effect of the genetic modification. The genetic makeup and environmental conditions can affect the composition and nutrient levels of corn. Furthermore, the differences observed are small and would not represent a difference that is nutritionally significant.

Combined USA and Canada

Data from all field trials were combined and analysed for significant differences between the genetically modified T25 corn line and its non-genetically modified counterpart. The results from this analysis are listed in Tables 4. The ranges for each component as listed in the USDA Handbook-8 (HB-8) are given.

Overall there were no significant differences in the proximate variables or minerals between genetically modified corn line T25 and its non-genetically modified counterpart. A significant difference was noted in the crude fat value which was higher in the genetically modified corn. There were also differences between several fatty acids: values were higher in the genetically modified corn for C16:1, C18:0, C18:2 and lower for C18:1. The only amino acid showing a significant difference

was lysine which was lower in the genetically modified corn than in the control line corn. The value for phosphorous was higher in genetically modified corn. Location was found to have a significant effect on some of these parameters. These differences are all small and are not considered to be biologically or nutritionally significant. Additionally, all nutrient values are similar to the values for USDA HB-8 or literature ranges for corn.

Table 2: Proximate, fatty and amino acid and mineral values for T25-2 and control grain grown in USA

Parameter[1]	Control	Transgenic	USDA HB-8[2]	Range[3]
Protein*	8.3 ± 0.6	10 ± 1.0	9.42 ± 0.89	9.0-11.2
Carbohydrate*	86.0 ± 0.8	84 ± 1.0	74.26	73.5
Fat	4.4 ± 0.1	4.6 ± 0.3	4.74 ± 0.91	4.1-4.8
Crude Fibre	2.6 ± 0.5	2.7 ± 0.6	2.90 ± 0.28	2.1-2.6
Ash	1.3 ± 0.2	1.5 ± 0.1	1.20 ± 0.14	1.4-2.0
C16:0	9.2 ± 0.2	9.3 ± 0.2	na	10.7-11.5
C18:0	2.5 ± 0.2	2.4 ± 0.2	na	2.2-4.1
C18:1	31.7 ± 0.8	31.8 ± 0.9	na	14-64; 24.5-27.
C18:2	54.3 ± 1.0	54.2 ± 1.3	na	19-71; 51.8-58.
C18:3*	1.0 ± 0.05	0.92 ± 0.04	na	0.5-2.0; 0.8-1.1
C20:0*	0.54 ± 0.01	0.51 ± 0.03	na	0.2
Calcium*	0.0051 ± 0.007	0.0141 ± 0.0007	0.007 ± 0.002	0.007- 0.05
Phosphorus*	0.286 ± 0.007	0.331 ± 0.001	0.210 ± 0.076	0.20-0.32
Alanine	0.53 ± 0.06	0.63 ± 0.1	0.61	na
Arginine*	0.28 ± 0.02	0.37 ± 0.05	0.41	na
Aspartic acid*	0.45 ± 0.04	0.55 ± 0.09	0.57	na
Cysteine	0.16 ± 0.02	0.18 ± 0.01	0.15	na
Glutamic acid	1.2 ± 0.1	1.3 ± 0.6	1.53	na
Glycine*	0.28 ± 0.02	0.32 ± 0.02	0.33	na
Histidine*	0.23 ± 0.02	0.27 ± 0.03	0.25	na
Isoleucine	0.20 ± 0.02	0.26 ± 0.06	0.29	na
Leucine	0.78 ± 0.09	0.98 ± 0.25	1.00	na
Lysine*	0.21 ± 0.01	0.25 ± 0.02	0.23	na
Methionine	0.17 ± 0.02	0.18 ± 0.03	0.17	na
Phenylalanine	0.30 ± 0.04	0.37 ± 0.09	0.40	na
Proline	0.56 ± 0.05	0.65 ± 0.2	0.71	na
Serine	0.35 ± 0.03	0.42 ± 0.07	0.39	na
Threonine*	0.26 ± 0.02	0.30 ± 0.04	0.31	na
Tryptophan	0.042 ± 0.008	0.050 ± 0.006	0.057	na
Tyrosine	0.12 ± 0.03	0.16 ± 0.05	0.33	na
Valine*	0.30 ± 0.02	0.37 ± 0.05	0.41	na

[1] Proximates and minerals expressed on a dry weight basis; fatty acids as percent of total lipids and adjusted for moisture. Amino acids reported as a % total protein.

[2] Values reported in Handbook 8, the United States Department of Agriculture (USDA), 1989. SD not available for fatty and amino acid values in the USDA HB-8. HB-8 lists values for fatty acid values adjusted for moisture.

[3] Wright, 1987 in Corn chemistry and technology, 1987, Watson SA and Ramstad PE (eds), American Association of Cereal Chemists, St. Paul, Minesota, USA; Ensminger *et al*, 1990; Macgregor, 1989; Perry 1988

*Significantly different from non-genetically modified counterpart.

Table 3: Proximate, fatty and amino acid and mineral values for T25-5 grain and control corn grown in Canada

Parameter[1]	Control	Transgenic	USDA HB-8[2]	Range[3]
Protein	10.3 ± 0.5	10.1 ± 0.5	9.42 ± 0.89	9.0-11.2
Carbohydrate	83. ± 0.5	83.7 ± 0.7	74.26	73.5
Fat	4.4 ± 0.2	4.7 ± 0.2	4.74 ± 0.91	4.1-4.8
Crude Fibre	2.6 ± 0.3	2.5 ± 0.3	2.90 ± 0.28	2.1-2.6
Ash	1.35 ± 0.05	1.41 ± 0.04	1.20 ± 0.14	1.4-2.0
C16:0	11.2 ± 0.4	11.8 ± 0.9	na	10.7-11.5
C18:0	1.9 ± 0.1	2.0 ± 0.1	na	2.2-4.1
C18:1*	29.0 ± 3.0	23.8 ± 0.7	na	14-64; 24.5-27.
C18:2*	55.0 ± 3.0	60.0 ± 2.0	na	19-71; 51.8-58.
C18:3	1.2 ± 0.2	1.2 ± 0.1	na	0.5-2.0; 0.8-1.1
C20:0*	0.50 ± 0.03	0.46 ± 0.02	na	0.2
Calcium	0.0042 ± 0.0006	0.005 0.001	0.007 ± 0.002	0.007- 0.05
Phosphorus	0.29 ± 0.03	0.30 ± 0.03	0.210 ± 0.076	0.20-0.32
Alanine	0.60 0.05	0.60 0.06	0.61	na
Arginine	0.32 0.04	0.32 0.03	0.41	na
Aspartic acid	0.54 0.06	0.50 0.05	0.57	na
Cysteine*	0.15 ± 0.01	0.16 ± 0.01	0.15	na
Glutamic acid	1.5 ± 0.1	1.4 ± 0.2	1.53	na
Glycine	0.29 ± 0.02	0.28 ± 0.03	0.33	na
Histidine	0.25 ± 0.02	0.23 ± 0.02	0.25	na
Isoleucine	0.27 ± 0.02	0.26 ± 0.03	0.29	na
Leucine	1.02 ± 0.07	1.00 ± 0.11	1.00	na
Lysine	0.23 ± 0.02	0.22 ± 0.02	0.23	na
Methionine	0.15 ± 0.02	0.16 ± 0.02	0.17	na
Phenylalanine	0.40 ± 0.03	0.38 ± 0.03	0.40	na
Proline	0.69 ± 0.07	0.65 ± 0.08	0.71	na
Serine	0.40 ± 0.04	0.39 ± 0.04	0.39	na
Threonine	0.30 ± 0.03	0.28 ± 0.03	0.31	na
Tryptophan	0.052 ± 0.006	0.0049 ± 0.003	0.057	na
Tyrosine	0.16 ± 0.01	0.16 ± 0.01	0.33	na
Valine	0.35 ± 0.03	0.34 ± 0.03	0.41	na

[1] Proximates and minerals expressed on a dry weight basis; fatty acids as percent of total lipids and adjusted for moisture. Amino acids reported as a % total protein.

[2] Values reported in Handbook 8, the United States Department of Agriculture (USDA), 1989. HB-8 lists fatty acid values adjusted for moisture only. Standard deviation not available for amino acid values.

[3] Wright, 1987 in Corn chemistry and technology, 1987, Watson SA and Ramstad PE (eds), American Association of Cereal Chemists, St. Paul, Minesota, USA. Ensminger *et al*, 1990; Macgregor, 1989; Perry 1988;

*Significantly different from non-genetically modified counterpart.

Table 4: Proximate, amino and fatty acid and mineral values for kernels from T25 and control corn grown in USA and Canada

Parameter[1]	Control	Transgenic	USDA HB-8[2]	Range[3]
Protein	10.18 ± 0.66	10.06 ± 1.09	9.42 ± 0.89	9.0-11.2
Carbohydrate	84.08 ± 0.66	84.09 ± 1.18	74.26	73.5
Fat*	4.39 ± 0.13	4.50 ± 0.12	4.74 ± 0.91	4.1-4.8
Crude Fibre	2.50 ± 0.36	2.37 ± 0.25	2.9 ± 0.28	2.1-2.6
Ash	1.34 ± 0.05	1.34 ± 0.05	1.20 ± 0.14	1.4-2.0
Moisture % H_2O	18.45 ± 3.29	20.77 ± 5.02	10.37 ± 3.28	7-23
C16:0*	11.13 ± 0.32	11.58 ± 0.51	na	10.7-11.5
C18:0*	2.00 ± 0.26	2.45 ± 0.15	na	2.2-4.1
C18:1*	29.22 ± 2.57	25.92 ± 0.69	na	14-64; 24.5-27.
C18:2*	55.05 ± 2.35	57.37 ± 1.37	na	19-71; 51.8-58.
C18:3	1.23 ± 0.16	1.20 ± 0.13	na	0.5-2.0; 0.8-1.1
C20:0	0.51 ± 0.03	0.51 ± 0.03	na	0.2
Calcium	0.0056 ± 0.0027	0.0072 ± 0.0026	0.007 ± 0.002	0.007- 0.05
Phosphorus*	0.3005 ± 0.0263	0.3200 ± 0.0221	0.210 ± 0.076	0.20-0.32
Alanine	0.61 ± 0.04	0.60 ± 0.06	0.61	na
Arginine	0.32 ± 0.04	0.30 ± 0.04	0.41	na
Aspartic acid	0.55 ± 0.05	0.50 ± 0.05	0.57	na
Cysteine	0.16 ± 0.02	0.16 ± 0.02	0.15	na
Glutamic acid	1.44 ± 0.13	1.38 ± 0.15	1.53	na
Glycine	0.30 ± 0.03	0.30 ± 0.05	0.33	na
Histidine	0.25 ± 0.01	0.24 ± 0.02	0.25	na
Isoleucine	0.26 ± 0.03	0.24 ± 0.04	0.29	na
Leucine	1.01 ± 0.09	0.95 ± 0.14	1.00	na
Lysine*	0.23 ± 0.02	0.21 ± 0.02	0.23	na
Methionine	0.17 ± 0.04	0.17 ± 0.02	0.17	na
Phenylalanine	0.40 ± 0.04	0.36 ± 0.04	0.40	na
Proline	0.70 ± 0.06	0.65 ± 0.07	0.71	na
Serine	0.41 ± 0.02	0.39 ± 0.03	0.39	na
Threonine	0.30 ± 0.02	0.28 ± 0.02	0.31	na
Tryptophan	0.05 ± 0.01	0.05 ± 0.00	0.057	na
Tyrosine	0.16± 0.01	0.15 ± 0.02	0.33	na
Valine	0.35 ± 0.04	0.32 ± 0.03	0.41	na

[1] Proximates and minerals expressed on a dry weight basis; fatty acids as percent of total lipids and adjusted for moisture. Amino acids reported as a % total protein.

[2] Values reported in Handbook 8, the United States Department of Agriculture (USDA), 1989. HB-8 lists fatty acid values adjusted for moisture only, no standard deviation available for amino acids.

[3] Wright, 1987 in Corn chemistry and technology, 1987, Watson SA and Ramstad PE (eds), American Association of Cereal Chemists, St. Paul, Minesota, USA. Ensminger *et al*, 1990; Macgregor, 1989; Perry 1988;

*Significantly different from non-genetically modified counterpart.

Analysis of GA treated corn

During the 1994 USA field trial of corn hybrid T25-2 at a site in Indiana, plants were also treated with glufosinate ammonium. The experimental design consisted of three treatments (T25-2 not treated with glufosinate ammonium, T25-2 treated with glufosinate ammonium and non-transgenic), with three or four replications. The treated T25-2 corn received one application of 400 gm glufosinate ammonium/hectare at growth stage V5. Grain was harvested at maturity. All compositional variables, with the exception of fatty acids, were adjusted for moisture prior to statistical analysis.

Plants were harvested at maturity and at least three representative samples from each site, for each genetic background, were analysed.

There were no significant differences (P=0.05) between the treated and untreated transgenic corn (Table 5). Thus, the application of glufosinate ammonium on the genetically modified corn line does not appear to have an effect on the final composition of the corn kernel.

There were some significant differences between either the treated or untreated transgenic corn to the non-transgenic counterpart, which are indicated in Table 6. Although statistically significant differences were seen between the transgenic and the non-transgenic counterpart, all means observed fall within reported literature ranges and are not nutritionally significant.

Table 5: Proximate, amino and fatty acid and mineral values for kernels from treated and untreated T25 corn and control corn grown in the USA

Parameter[1]	Control	T25-2 untreated	T25-2 GA-treated	USDA HB-8[2, 4]	Range[3]
Protein*	6.81 ± 0.50	7.87 ± 1.15	9.22 ± 0.39	9.42 ± 0.89	9.0-11.2
Carbohydrate*	74.64 ± 2.29	73.50 ± 1.53	71.43 ± 0.40	74.26	73.5
Fat	3.76 ± 0.09	3.93 ± 0.20	4.20 ± 0.13	4.74 ± 0.91	4.1-4.8
Crude Fibre	2.60 ± 0.29	2.70 ± 0.29	2.43 ± 0.21	2.9 ± 0.28	2.1-2.6
Ash*	1.02 ± 0.10	1.21 ± 0.12	1.15 ± 0.06	1.20 ± 0.14	1.4-2.0
Moisture % H_2O	13.77 ± 2.51	12.72 ± 1.77	14.00 ± 0.33	10.37 ± 3.28	7-23
C16:0	9.11 ± 0.24	9.16 ± 0.21	8.88 ± 0.06	na	10.7-11.5
C18:0*	2.63 ± 0.02	2.24 ± 0.17	2.15 ± 0.03	na	2.2-4.1
C18:1	32.27 ± 0.53	31.37 ± 1.10	32.28 ± 0.31	na	14-64; 24.5-27.
C18:2	53.50 ± 0.43	54.83 ± 1.45	54.32 ± 0.18	na	19-71; 51.8-58.
C18:3*	1.03 ± 0.04	0.94 ± 0.03	0.88 ± 0.01	na	0.5-2.0; 0.8-1.1
Alanine	0.49 ± 0.04	0.55 ± 0.08	0.66 ± 0.06	0.61	na
Arginine*	0.26 ± 0.01	0.36 ± 0.06	0.33 ± 0.03	0.41	na
Aspartic acid*	0.43 ± 0.03	0.50 ± 0.08	0.60 ± 0.06	0.57	na
Cysteine	0.15 ± 0.01	0.17 ± 0.02	0.16 ± 0.01	0.15	na
Glutamic acid	1.13 ± 0.10	0.24 ± 0.29	1.60 ± 0.15	1.53	na
Glycine*	0.27 ± 0.14	0.32 ± 0.03	0.32 ± 0.02	0.33	na
Histidine*	0.21 ± 0.01	0.26 ± 0.04	0.28 ± 0.02	0.25	na
Isoleucine	0.19 ± 0.03	0.21 ± 0.05	0.25 ± 0.01	0.29	na
Leucine	0.73 ± 0.09	0.81 ± 0.17	1.03 ± 0.05	1.00	na
Lysine*	0.20 ± 0.01	0.25 ± 0.02	0.26 ± 0.01	0.23	na
Methionine	0.15 0.005	0.15 ± 0.02	0.15 ± 0.01	0.17	na
Phenylalanine*	0.28 ± 0.03	0.33 ± 0.07	0.41 ± 0.01	0.40	na
Proline	0.53 ± 0.05	0.54 ± 0.16	0.69 ± 0.06	0.71	na
Serine	0.33 ± 0.03	0.37 ± 0.06	0.44 ± 0.04	0.39	na
Threonine*	0.24 ± 0.02	0.28 ± 0.04	0.30 ± 0.03	0.31	na
Tryptophan	0.040 ± 0.008	0.050 ± 0.008	0.047 ± 0.006	0.057	na
Tyrosine*	0.10 ± 0.01	0.14 ± 0.05	0.14 ± 0.00	0.33	na
Valine	0.30 ± 0.03	0.34 ± 0.05	0.35 0.03	0.41	na

[1] Proximates, amino acids and minerals expressed on a dry weight basis; fatty acids as percent of total lipids and adjusted for moisture. Amino acids reported as a % total protein.

[2] Values reported in Handbook 8, the United States Department of Agriculture (USDA), 1989. HB-8 lists fatty acid values adjusted for moisture only, no standard deviation available for amino acids.

[3] Wright, 1987 in Corn chemistry and technology, 1987, Watson SA and Ramstad PE (eds), American Association of Cereal Chemists, St. Paul, Minesota, USA. Ensminger *et al*, 1990; Macgregor, 1989; Perry 1988;

*Either the treated or non-treated genetically modified corn was significantly different from non-genetically modified counterpart – note: there were no significant differences between the treated and untreated genetically modified corns.

Levels of anti-nutrients

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

Ability to support typical growth and well-being

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 re-assurance 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 corn line T25, the extent of the compositional and other data evaluated is considered adequate to establish the safety of the food. Nonetheless, an animal feeding study to compare the wholesomeness of corn line T25 with conventional corn hybrids was performed. Although not considered essential for establishing safety in this instance, this animal feeding study has been reviewed as additional supporting data.

Study evaluated:

Leeson D (1996). The effect of glufosinate resistant corn on the growth of male broiler chickens. Performing laboratory: Department of Animal and Poultry Sciences, University of Guelph, Ontario, Canada. Report No. C-5-96I.

Two hundred and eighty commercial strain Ross x Ross male broiler chickens were weighed and allocated at random to 1 of 2 treatment groups, replicated 4 times, 35 birds per replicate. Birds were reared on 1 of 2 diets, *ad libitum*; a commercial corn hybrid or genetically modified corn line T25. Each diet was a conventional corn-soybean type, prepared for starter, grower and finisher periods. Birds were fed starter diets to 18 days, grower diets from 18–32 days, and finisher diets from 32–42 days of age. At 18, 32 and 42 days, feed intake was measured and all birds weighed individually. All occurrences of mortality were submitted for post mortem examination. On day 42, 8 birds were randomly selected from each group for processing.

Variables considered for analysis were initial body weight; 18, 32 and 42 day body weight; 0–18, 18–32 and 32–42 day body weight gain, feed intake; and feed intake:body weight gain. Carcass characteristics considered were chilled carcass weight; abdominal fat pad weight; total deboned breast meat yield and abdominal fat pad as a percent of carcass weight; and deboned breast meat yield as a percent of carcass weight. Percent mortality over the experimental period was calculated. Significance was accepted at $P<0.05$.

The source of corn in the starter, grower and finisher diets had no effect on body weight, feed intake, feed intake:body weight gain or percent mortality over the experimental period ($P>0.05$). The mortality rate of 7.14 ± 5.47% was normal for this fast-growing strain of bird, with normal values being 5 – 8%. Carcass characteristics measured and calculated were unaffected by source of corn in the experimental diets.

Herbicide-tolerant corn was comparable in feeding value for 0–42 day broilers, relative to commercially available corn. The results indicate that the nutritive value of the herbicide-tolerant corn hybrid is equivalent to a commercially available corn hybrid and also supports the conclusion of the compositional analyses that there are no biologically significant differences between corn line T25 and other commercial varieties of corn.

Conclusion

The nutritional qualities of glufosinate ammonium tolerant corn line T25 were determined by compositional analyses of the major components of the kernels and these were found to be comparable in all respects to the conventional corn lines.

There is a long history of safe use of corn. Based on the data evaluated in this assessment, grain derived from corn line T25is nutritionally and compositionally comparable to that from conventional corn and is not considered to pose a risk to human health and safety.

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