# FOOD DERIVED FROM GLYPHOSATE - TOLERANT CORN LINE GA21

## A Safety Assessment

TECHNICAL REPORT SERIES NO. 7

**AUSTRALIA NEW ZEALAND FOOD AUTHORITY**
November 2001

© Australia New Zealand Food Authority 2001
ISBN 0 642 34531 7
ISSN 1446-4977
Published November 2001

This work is copyright. Apart from any use as permitted under the *Copyright Act 1968*, no part may be reproduced by any process without prior written permission from Australia New Zealand Food Authority (ANZFA). Requests and inquiries concerning reproduction and rights should be addressed to the Information Officer, ANZFA, PO Box 7168, Canberra BC, ACT 2610.

An electronic version of this work is available on the Australia New Zealand Food Authority (ANZFA) website at http://www.anzfa.gov.au. This electronic version may be downloaded, displayed, printed and reproduced in unaltered form only for your personal, non-commercial use or use within your organisation.

ANZFA Australia
PO Box 7186
Canberra BC ACT 2610
Australia
Tel +61 2 6271 2241
Fax +61 2 6271 2278
Email info@anzfa.gov.au

ANZFA New Zealand
PO Box 10599
Wellington
New Zealand
Tel + 64 4 473 9942
Fax +64 4 473 9855
Mail nz.reception@anzfa.gov.au

## TABLE OF CONTENTS

## SUMMARY

Food derived from glyphosate-tolerant corn line GA21 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*

Glyphosate-tolerant corn line GA21 has been developed to provide growers with a crop that is tolerant to applications of the broad spectrum herbicide, glyphosate. This trait has been introduced into corn plants by the addition of a modified corn gene encoding the EPSPS protein, a key enzyme in the biosynthesis of aromatic amino acids in plants and microbes. The modification alters the sensitivity of the enzyme to glyphosate, resulting in sufficient enzyme activity to allow the plant to function in the presence of the herbicide.

As well as the mEPSPS gene, other DNA elements transferred into the corn include the rice actin promoter and intron, which have been shown to direct constitutive protein expression in corn, together with an optimised chloroplast transit peptide sequence to direct translocation of the mEPSPS protein to chloroplasts, where the protein is functionally active. The NOS 3' untranslated region is present, also for regulatory purposes, providing the appropriate eukaryotic polyadenylation signal. Because a purified fragment of DNA was used in the transformation, no extraneous bacterial genes, including laboratory marker genes, were transferred.

### *General safety issues*

There is a comprehensive set of analytical data for the safety assessment of the transgenic corn. There is only one new protein, namely the mEPSPS enzyme, produced by the genetic modification to the corn plants. Despite some amino acid changes, the mEPSPS shows more than 99.3% homology with the conventional corn EPSPS enzyme. The new protein is present in corn grain at levels approximately ten times that of the endogenous corn protein, however the family of EPSPS proteins are ubiquitous in plant and microbial food sources which are already part of human diets.

### *Toxicological issues*

Corn has undergone substantial genetic breeding by conventional methods over many centuries and has been safely consumed as food and feed for thousands of years. As the changes to the corn enzyme involve substitutions with standard amino acids common to all proteins of biological origin, and do not alter the functional properties of the enzyme, the mEPSPS protein is not considered to be inherently toxic. This was supported by the results of an acute toxicity study in mice, where animals given a variable single dose of the purified mEPSPS protein showed no clinical signs of toxicity and continued to grow normally for the duration of the 14 day study.

Similarly, there is no evidence to suggest that the transgenic corn would be more likely to cause allergies than the conventional counterpart. The mEPSPS lacks similarity to known allergens and protein toxins, is rapidly degraded in simulated digestive systems and occurs at low levels in the protein fraction of the grain.

***Nutritional issues***

The results of extensive compositional analyses on both treated and untreated plants demonstrate that the levels of the important components in corn grain (protein, total fat, carbohydrate, ash, fibre, fatty acids, amino acids and moisture) and the minerals calcium and phosphorus in this transgenic line are comparable to the non-transgenic control and to available published literature ranges. The safety of the mEPSPS protein to humans is therefore established by consideration of the results obtained from the biochemical and genetic analyses that demonstrate its similarity to the conventional form of the enzyme in terms of its functional properties.

***Conclusion***

Based on currently available data, food derived from glyphosate-tolerant corn line GA21 is comparable to conventional corn in terms of its safety and nutritional adequacy.

# FOOD DERIVED FROM GLYPHOSATE-TOLERANT CORN LINE GA21

## A SAFETY ASSESSMENT

## INTRODUCTION

A safety assessment has been conducted on food derived from corn that has been genetically modified to be tolerant to the herbicide, glyphosate. The modified corn is referred to as glyphosate-tolerant corn line GA21.

Glyphosate is the active ingredient of the herbicide Roundup® which is used widely as a non-selective agent for controlling weeds in primary crops. The mode of action of glyphosate is to specifically bind to and block the activity of 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), an essential enzyme involved in the biosynthesis of aromatic amino acids in all plants, bacteria and fungi. Biochemical studies on the EPSPS enzyme from a variety of different species have shown that a natural variation in glyphosate binding affinity exists, particularly across bacterial species (Schultz *et al*. 1985). Further studies on bacterial and plant EPSPS enzymes demonstrated that sequence changes at the active site of the enzyme, a highly conserved region across species, could alter substrate and inhibitor binding properties (Padgette *et al.*, 1991). Tolerance to glyphosate in plants can therefore be achieved by introducing a version of the EPSPS gene producing a protein with a reduced binding affinity for glyphosate, thus allowing the plant to function normally in the presence of the herbicide.

In glyphosate-tolerant corn line GA21, the glyphosate-tolerant trait is generated in the plants through specific changes to the corn (*Zea mays*) gene which results in the production of a modified EPSPS enzyme, the so-called mEPSPS protein. The modification produces an enzyme that is less sensitive to glyphosate, compared with the unmodified corn enzyme, and thus imparts glyphosate tolerance to the whole plant. The mEPSPS protein exhibits more than 99.3% amino acid homology with the conventional corn EPSPS protein.

Corn is used in the manufacture of breakfast cereals, baking products, extruded confectionery and corn chips. Corn starch is used by the food industry for the manufacture of dessert mixes and canned foods. A small proportion (400 tonne in 1995/96) of corn products is imported in the form of high-fructose corn syrup, according to market demand.

## DESCRIPTION OF THE MODIFICATION

Studies evaluated:

D.A. Dixon, L.A. Turner, T.A. Dowey, T.C. Lee and J.N. Leach, 1997. Molecular Analyses of Roundup Ready Corn Line GA21. Performing Laboratory: Monsanto Company, Molecular Analysis Centre, Report No. MSL-15205, Study 97-01-46-10.

R.P. Lirette, D.A. Dixon, S-Z. Pang, L. Albee, R. Krieb, C. Hironaka, J. Astwood and R.S. Sidhu,1998. Additional molecular characterisation of Roundup Ready® corn line GA21. Performing Laboratory: Monsanto Life Sciences Company, Report No. MSL-15335, Study 97-01-46-12.

### Methods used in the genetic modification

Corn line GA21 was generated by transformation of corn (*Zea mays*) using a particle acceleration transformation system. This method of transformation allowed for a specific linear fragment of DNA incorporating only the gene of interest together with essential controlling elements to be transferred to the plant. Since the introduced DNA contains a gene encoding for herbicide tolerance (in this case, the mEPSPS gene), the plant cells are grown in the presence of glyphosate and only those cells which carry the DNA modification continue to grow.

### Function and regulation of the novel genes

A specific 3.4 kb DNA fragment from plasmid pDPG434 was purified by agarose gel electrophoresis and subsequently introduced into embryogenic corn cells. The purified fragment, referred to as the gene cassette, contained only the modified corn EPSPS gene fused to an optimised chloroplast transit peptide sequence and controlling DNA elements essential for expression in plant cells (see below). The mEPSPS gene is under the regulation of the rice actin promoter and rice actin intron, and the 3' untranslated region of the nopaline synthase gene (NOS 3') from the Ti plasmid of *Agrobacterium*.

Although plasmid pDPG434 contained other bacterial genes and controlling sequences for selection and replication in the laboratory, these sequences were not contained within the purified fragment used in the transformation and therefore are not present in the plant.

*Actin Promoter*

To direct expression of the inserted gene, a rice actin promoter was used (McElroy *et al*, 1990.). The promoter region is comprised of a 1.37 kb DNA sequence corresponding to the 5' region of the rice actin 1 gene, containing the promoter site and first intron. This plant derived promoter provides for constitutive expression in all parts of the corn plant.

*mEPSPS gene*

The modified EPSPS gene was produced by cloning the wildtype EPSPS gene from corn (*Zea mays*) in plasmid pDPG434 and introducing specific changes to the DNA using standard *in vitro* techniques (see Padgette *et al*., 1991). Although full details of

the exact nature of the changes have been provided to ANZFA for assessment, at the request of the applicant, this information has been deemed confidential commercial information. The specific changes to regions of the corn gene result in the production of the mEPSPS protein with enzymatic activity that is glyphosate-insensitive relative to the unmodified form of the enzyme.

*Chloroplast transit peptide*

Natural EPSPS enzyme is located within chloroplasts, the site of aromatic amino acid biosynthesis in plant cells. As for many proteins with subcellular locations, newly synthesised preproteins are directed to a particular organelle by a transit peptide usually at one end of the mature protein. Following delivery to the organelle, the short transit peptide is cleaved from the mature protein and is rapidly degraded (della-Cioppa *et al*. 1986).

To direct the mEPSPS protein to the chloroplast of plant cells, the mEPSPS gene is fused to chloroplast transit peptide (CTP) sequences, to generate an optimised transit peptide (OTP) (Lebrun et al., 1996). These transit sequences are derived from plant sequences isolated from corn and sunflower ribulose 1,5 –bisphosphate carboxylase oxygenase (RuBisCo). The mEPSPS gene with its OTP sequence is approximately 1.7 kb in size. The entire deduced amino acid sequence of the mEPSPS preprotein including the 125 amino acids of the OTP was provided. The fusion of the mEPSPS with the OTP results in an additional amino acid (methionine) at the amino-terminal end of the mature mEPSPS protein, following cleavage of the transit peptide.

*NOS 3' untranslated region*

The NOS 3' untranslated sequence is derived from the common soil bacterium *Agrobacterium tumefaciens*. It is a region of DNA copied from the bacterial nopaline synthase gene (*nos*) that operates in plants by providing the polyadenylation signal for stable expression.

**Characterisation of the genes in the plant**

*Molecular characterisation of corn line GA21*

Molecular characterisation of the integrated DNA present in Roundup Ready corn line GA21 was performed using untransformed corn DNA and plasmid pDPG434 as reference material. Using Southern blot analyses, genomic DNA from the transformed line was analysed for the number of sites of insertion of the plasmid DNA into the corn genome and the integrity of the genetic elements contained within the inserted DNA.

The results of experiments using several different probes specific for the purified DNA fragment showed that a single insertion event has resulted in the introduction of a segment of DNA of approximately 18.5 kb. Further analysis demonstrated that the inserted segment contained three complete copies in tandem of the plasmid fragment used in the transformation plus an incomplete copy. The partial copy has been demonstrated to include the rice actin promoter and a truncated mEPSPS gene which lacks the NOS 3'untranslated region.

Further evidence in support of this molecular characterisation was provided by the results of a Western blot analysis performed in order to assess the equivalence of the modified EPSPS protein produced by corn line GA21 to that expressed in bacteria in the laboratory. This experiment showed that only one immunoreactive protein of the expected apparent molecular weight (approx. 47 kD) is found in crude extracts of the transformed corn tissue.

*Characterisation of the 5' and 3' ends of the inserted DNA in GA21 corn*

The above characterisation (see 2.3.1) of the modification in line GA21 including the results from Southern blot analyses, allowed predictions of the genetic makeup of the inserted DNA based on size and sensitivity to restriction enzyme digestion. These reports established that the inserted DNA of line GA21 contains, as a single insert, three copies of the mEPSPS gene cassette, plus an incomplete copy consisting of the rice actin promoter, the optimised transit peptide and a truncated mEPSPS sequence without the NOS 3' untranslated region. However, these studies did not provide molecular detail about the ends of the inserted DNA, particularly where these are adjacent to flanking corn DNA. With regard to the direction of transcription (and hence protein synthesis) the starting end of the DNA segment is denoted as the 5' end, with the opposite end denoted as the 3' end.

Testing and experiments in the laboratory allowed for cloned segments of the corn GA21 genomic DNA to be analysed in greater detail by direct DNA sequencing. The DNA sequence data was verified by polymerase chain reaction (PCR) analysis of genomic DNA from the GA21 corn. Sequence analysis showed a truncated rice actin promoter at the 5' end of the GA21 insert. The truncated promoter contained the last 148 base pairs (bp) of the 3' end of the rice actin promoter including the rice actin intron. This partial rice actin promoter is expected to be functional and enable the production of the full length mEPSPS protein, based on the detailed characterisation of the rice actin promoter in the published literature (McElroy et al., 1990).

Detailed analysis at the 3' end of the GA21 insert established the presence of the full length rice actin promoter, the optimised transit peptide and, as expected, a truncated mEPSPS gene, but lacking the NOS polyadenylation signal. This truncated sequence contained the first 289 bp of the mEPSPS coding sequence, terminating in a translational stop codon. Northern blot analysis showed that, whereas a stable transcript was detected for the complete mEPSPS gene cassette, the truncated mEPSPS sequence does not produce a detectable transcipt. Further evidence that the truncated gene does not express protein was provided by Western blot analysis which showed that only a single band corresponding to the full length mEPSPS protein was expressed in GA21 corn.

The DNA sequence analysis of the 3' end of the GA21 inserted segment also showed the presence of a partial mEPSPS cassette containing the rice actin promoter truncated before the start of the rice actin intron, and fused at the 3' end to corn genomic DNA. Based on DNA sequence data, two putative overlapping open reading frames, ORF-1 (97 amino acids) and ORF-2 (19 amino acids) were identified. However, Northern blot analysis using poly (A+) RNA prepared from leaf tissue of corn line GA21 demonstrated that there was no detectable RNA transcript of this region. This finding

is supported by detailed published studies on the rice actin promoter elements which report that in the absence of the intron, protein is not produced (McElroy et al., 1990).

Despite the lack of evidence that the region of proximal corn DNA is transcribed, further investigations were carried out. The putative amino acid sequences corresponding to ORF-1 and ORF-2 were compared to all known allergens and toxins present in public domain databases. No sequence similarity was found for either ORF-1 or ORF-2 when the comparisons were done according to established criteria for allergen screening (Metcalfe et al., 1996). Similarly, neither ORF-1 nor ORF-2 encoded amino acid sequences meeting established criteria suggesting homology to protein toxins (Doolittle, 1990).

The database searches indicated that the DNA sequences corresponding to ORF-1 and ORF-2 were found to be homologous to highly repetitive DNA regions commonly found in the intergenic regions separating functional genetic loci in corn which represent more than 50% of the corn genome (San Miguel et al., 1996). This is further evidence that the putative ORFs associated with the 3' proximal corn genomic DNA at the integration site in corn line GA21, would not produce any protein.

The results of all of these analyses confirm the characterisation of the inserted DNA. Glyphosate-tolerant corn line GA 21 contains a single inserted segment of DNA which expresses only the full length mEPSPS protein.

**Stability of the genetic changes**

Progeny from successive backcrossing of the transgenic line were tested for five generations and the data indicate that the inserted DNA is stably integrated into the corn genome. Analysis of the progeny from one generation of self pollination of the fifth generation of backcrossed plants (BC5F2) also demonstrates the stability of the modification according to Mendelian inheritance. These results are consistent with the genetic and molecular analyses described above.

**Conclusion**

Based on the detailed molecular characterisation, glyphosate-tolerant corn line GA21 contains, as a single insert, four functional mEPSPS gene cassettes plus a truncated mEPSPS cassette that does not produce a detectable RNA transcript. The only protein expressed from the inserted DNA is the full length mEPSPS protein.

## GENERAL SAFETY ISSUES

Studies evaluated:

T. C. Lee and M. Bailey, 1996. Assesment of the equivalence of CP4 EPSPS protein produced in *Escherichia coli* and in several insect protected , Roundup Ready® and insect protected/Roundup Ready® corn lines. Performing Laboratory: Monsanto Company, CEREGEN, Report No. MSL-14746, Study 95-01-50-05.

Glyphosate-tolerant corn line GA21 has been assessed according to ANZFA's paper entitled 'Guidelines for the safety assessment of foods to be included in Standard A18

– Food Produced Using Gene Technology' relating to Group D foods. This process is applicable to foods derived from a plant or animal that contains new or altered genetic material (ANZFA 1999).

**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, most consumers are not aware that corn is an important source of the sweeteners, starches, oil and alcohol used in many foods, beverages and numerous other products.

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.

**Nature of novel protein**

As part of the safety assessment of glyphosate-tolerant corn line GA21, the assessment examines the expressed products of the introduced genes and considers the levels of new protein in the grain. In this line, the pre-mEPSPS protein is the only expressed protein product from the inserted gene cassette, the other DNA elements being controlling sequences. Under the regulation of the rice actin promoter, the mEPSPS protein is expected to occur throughout the whole plant, since this promoter has been shown to drive constitutive expression in genetically modified corn.

Since the EPSPS protein is naturally present in plants, bacteria and fungi as part of the basic biochemical makeup of the organism, several scientific studies have compared the amino acid sequences and catalytic properties of the enzyme from a wide variety of different sources (for example, see Schultz *et al.*, 1985). Data from these studies shows differences in amino acid sequence of the enzyme from different species, including bacteria and fungi, which directly alters the sensitivity to glyphosate of these naturally occurring forms of the enzyme. Such information promoted further

study of the catalytically important amino acid residues of this key metabolic enzyme and provided the scientific background material for development of the mEPSPS in this application. Therefore, with respect to this enzyme in the environment, considerable sequence variation exists across species, and several naturally occurring versions exhibit a concomitant range of natural tolerance levels to the herbicide glyphosate.

The mEPSPS gene has been completely sequenced and encodes a protein of 47.4 kDaltons consisting of a single polypeptide of 445 amino acids. The amino acid sequence identity between the modified enzyme and the wildtype enzyme from corn is greater than 99.3%.

The new protein was expressed in *E. coli* and purified to allow characterisation of its enzymatic properties. Kinetic and enzyme activity analyses indicate that the mEPSPS enzyme interacts with the normal EPSPS substrates, shikimate-3-phosphate and phosphoenolpyruvate, similarly to the wildtype corn EPSPS enzyme.

**Expression of the novel protein in the plant**

Studies evaluated:

T.C. Lee *et al*., 1997. Assessment of the Equivalence of Modified Maize 5-Enolpyruvylshikimate-3-phosphate Synthase (mEPSPS) Produced in *Escherichia coli* and in the Roundup Ready® Maize Line GA21.

To verify expression, levels of the new protein were evaluated in forage and grain samples collected from five field locations in the U.S. during the 1996 growing season, using an Enzyme Linked Immunosorbent Assay (ELISA). As for these and other tests, corn plants identified by PCR as negative segregants were used as controls.

The polyclonal antibody used in the assay system was also an appropriate reagent for the detection of wildtype corn EPSPS as well as the mEPSPS protein, however the expression of wildtype EPSPS was below detectable levels in the grain of the control samples. It is known that the expression of endogenous EPSPS in plant tissues is very low relative to microorganisms (Mousdale and Coggins, 1984). For treated transgenic GA21 grain, the mean expression of EPSPS protein, representing the sum of the endogenous and modified corn EPSPS expression levels, was 4.6 μg/g fresh weight (range was 1.7-7.4 μg/g fwt). The quantitation limit of the EPSPS ELISA assay was approximately 0.8 μg/g for grain. The results of this assay showed that the expression of mEPSPS protein in corn line GA21 was at least one order of magnitude greater than that of the wildtype EPSPS expressed in the non-transgenic control.

Western blot analysis was used to further assess the expression of the mEPSPS in the modified corn. This technique provides for high specificity and also allows for comparison of the apparent size (molecular weights) of proteins with immunological cross-reactivity present in complex mixtures of crude protein extracts from corn. In particular, this procedure tested for the presence of any related proteins with cross-reactivity with the EPSPS specific antibody, but not of the predicted size, and therefore allowed for the detection of unexpected fusion proteins.

The results from the Western blot procedure indicated the presence, in the GA21 transgenic line, of a single protein band of equivalent molecular weight to the expected mEPSPS as expressed *in vitro* in the laboratory. This procedure also confirmed that the expression level of mEPSPS in the corn grain was at least ten times greater than that of the endogenous corn EPSPS enzyme.

In summary, the data obtained from both the ELISA and Western blot procedures indicate that the mEPSPS is expressed in corn line GA21 at greater than 10 fold the levels of the endogenous EPSPS in the non-transgenic control, and that the protein produced in the transgenic plant is equivalent to the predicted size according to its characterisation *in vitro*. Furthermore, the absence of any other immunoreactive bands of unexpected size is evidence that the transformation has resulted in the expression of only the mEPSPS protein of the predicted size in the modified plants.

*Conclusion*

One new protein is expressed in corn line GA21, namely the modified corn EPSPS, which carries some specified amino acid changes compared to the wildtype enzyme. Although several methods of analysis showed that the mEPSPS is expressed in the edible grain from the plant at levels approximately ten times higher than endogenous EPSPS expression levels, this is not considered to be a safety issue due to the prevalence of this family of plant and microbial proteins in the human diet.

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

The human health considerations in this regard depend 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 Expert Consultation which looked at strategies for assessing the safety of foods produced by biotechnology (WHO, 1991). It was concluded by that consultation 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 cells in the human digestive tract is with 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). There have been concerns expressed, however, that there could be horizontal gene transfer of antibiotic resistance genes from ingested food to microorganisms present in the human digestive tract and that this could compromise the therapeutic use of antibiotics.

In relation to the transfer of novel genetic material from genetically modified food to human cells via the digestive tract, this is extremely unlikely to occur. In considering

[1] Food and Agriculture Organisation

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.

The gene cassette in this modification includes a promoter element from another edible plant, the rice actin promoter, which has been shown to give rise to protein expression in corn plants. This eukaryotic promoter is necessary for expression of the new protein, mEPSPS. However, this element is not functional in prokaryotic gut microorganisms, as critical DNA sequences are not recognised by the protein expression machinery of bacteria, including those normally present in mammalian intestines.

Of significance for this application, the method of transformation allowed for a gel purified specific fragment of plasmid pDPG434 to be introduced into the corn cells, a process which effectively excluded the presence of antibiotic resistance marker genes and other bacterial genetic elements present elsewhere in the whole plasmid. Consequently, no extraneous DNA sequences such as marker genes were ever introduced into this plant line.

### Conclusion

There is no biological potential for the transfer of novel genetic material from corn line GA21 to intestinal microorganisms, as a result of the genetic modification. The DNA sequences which give rise to protein expression in the plant are not functional in prokaryotes. Furthermore, microorganisms including bacteria and fungi contain an endogenous EPSPS gene and corresponding protein product not unlike the version produced by the transgenic corn. Due to the variety of naturally occurring EPSPS sequences across species in the environment, there is a natural array of organisms exhibiting a potential for glyphosate tolerance.

## TOXICOLOGICAL ISSUES

### Levels of naturally occurring toxins

Over 72% of the corn kernel is composed of starch, with smaller amounts of protein, oil and other nutritionally valuable substances. There are no known naturally occurring toxins in corn. While mycotoxins can be detected in corn, these are metabolites produced by fungi that grow on corn kernels as a result of production or storage under adverse conditions. They are not a natural component of sound corn.

### Potential toxicity of novel protein

The detailed protein expression analyses have demonstrated that the only new protein present in corn line GA21 is the mEPSPS enzyme. The mEPSPS gene has been

completely sequenced and encodes a 47.4 kDa protein consisting of a single polypeptide of 445 amino acids. The modified corn mEPSPS protein shows high amino acid sequence homology to the wildtype corn EPSPS enzyme (99.3%) as well as to other EPSPS enzymes found in common food crops (for example, soybean and tomato) that have a long history of safe human consumption, or that are present in fungal and microbial food sources such as Baker's yeast (*Saccharomyces cerevisiae*) or *Bacillus subtilis*. Thus, notwithstanding the minor change of several amino acids, this protein is a member of a family of closely related proteins from plants and microbes that are commonly found in human foods.

The amino acid sequence of the mEPSPS protein was compared to that of known protein toxins listed in the PIR, SwissProt, EMBL and GenBank genetic databases. Based on these computer searches, no evidence for any similarity to known protein toxins was found. The specific amino acid changes in the mEPSPS protein, being standard substitutions with common amino acids that comprise all proteins, are unlikely to result in a protein with toxic properties.

As a further test for potential toxicity, the applicant conducted an acute oral toxicity study of the mEPSPS protein in young laboratory mice (approximately 7.5 weeks of age).

*Acute oral toxicity study in mice*

Studies evaluated:

T.C. Le *et al*., 1997. Preparation and Confirmation of Doses for an Acute Oral Toxicity Study (ML-97-195) with Modified Maize 5-Enolpyruvylshikimate-3-Phosphate Synthase (mEPSPS) Protein in Albino Mice.

M.W. Naylor, 1997. Acute Oral Toxicity Study with Modified Maize 5-Enolpyruvylshikimate-3-phosphate Synthase (mEPSPS) Protein in Albino Mice.

Supplies of the mEPSPS protein required for the oral toxicity study in animals were produced in *E. coli* in the laboratory. The protein was partially purified and in a separate study, the applicant conducted experiments to formulate and characterise the mEPSPS prior to its use as a test substance in the toxicity study. Confirmation of the mEPSPS protein concentration, integrity and functional activity was experimentally determined by a series of analyses including a total protein assay, densitometry of colloidal blue stained SDS-PAGE gels, Western blot analysis, and enzyme activity assay. From this information, the applicant determined an appropriate formulation and dose range of mEPSPS suitable for use as the test substance.

In the toxicity study, the mEPSPS was administered by a single oral gavage to ten male and ten female CD-1 mice, at target doses of 5, 15 and 50 mg/kg in a constant volume. Using the results of the equivalence study above, this corresponded to actual doses of 3.7, 11.8 and 45.6 mg/kg respectively. Based on the highest anticipated human exposure of 0.06 mg mEPSPS /kg bw/day calculated for children, 1-6 years of age (in the U.S.), the applicant claims that the highest dose of 45.6 mg mEPSPS/kg bw is at least 500-fold higher than the likely human exposure (Holden, 1997).

A control group of ten mice/sex was administered only the carrier substance used above, at the same delivery volume as the test substance. An additional control group of ten mice/sex was administered Bovine Serum Albumin (BSA) in the same carrier substance at the highest target dose (50 mg/kg) and also in the same delivery volume. At defined stages throughout the duration of the study, clinical observations were performed for mortality and signs of toxicity, and body weights and food consumption measured. At the termination of the study (day 13-14), animals were sacrificed, examined for gross pathology and numerous tissues were collected. However, no organs were weighed and no tissues were examined microscopically.

The results of the study showed no statistically significant differences in group mean body weights, cumulative weight gains or food consumption in either males or females at any level of either the BSA control or test material, when compared with the respective carrier control group. All animals survived to the end of the study, and there were no clinical signs observed that could be related to the test material. A unilateral corneal opacity was noted in one male mouse at the high dose level of the test material, but this finding was not considered to be treatment related.

In conclusion, there was no evidence of toxicity in mice following a single oral dose of 45.6 mg/kg mEPSPS protein.

**Potential allergenicity of novel protein**

| Studies evaluated: |
| --- |
| J.D. Astwood, 1997. Modified Maize 5-Enolpyruvylshikimate-3-Phosphate Synthase (mEPSPS) has no significant Sequence Similarity to known Allergens and Toxins. |

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.

The mEPSPS gene was derived from corn which is not regarded as an allergenic food (Wright, 1987). No known allergens have ever been confirmed to be present in corn. At the protein level, the mEPSPS enzyme exhibits 99.3% amino acid homology with the wildtype corn EPSPS enzyme which is commonly found in food. The mEPSPS protein is present at low levels in the grain, at approximately 0.01% of the total protein in grain from transgenic corn line GA21.

As an indicator of allergenic potential, the amino acid sequence of the introduced mEPSPS protein was compared with amino acid sequences of known allergens available on public protein databases. Based on published scientific information about the common molecular features of known protein allergens, a sequence match of at least eight contiguous identical amino acids is considered to be a significant degree of homology. When the appropriate comparisons were made, no biologically or immunologically significant sequence similarities were observed between the mEPSPS and at least 219 allergen sequences. It is therefore concluded that the mEPSPS gene introduced into corn does not encode a known allergen and that the

mEPSPS protein does not share immunologically significant amino acid sequences with known allergens.

*Digestibility of the mEPSPS*

| Studies evaluated: |
| --- |
| R.S. Sidhu *et al*. 1997. Assessment of the Digestability of Modified Maize 5-Enolpyruvylshikimate-3-phosphate Synthase (mEPSPS) Protein *in vitro* Using Mammalian Digestive Fate Models. |

The biochemical profile of the mEPSPS enzyme also provides a basis for allergenic assessment when compared to known protein allergens. Protein allergens must 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 allergenic response.

The purpose of this study was to assess the digestibility of mEPSPS protein *in vitro* using simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) as mammalian digestion models. The method of preparation of the simulated mammalian gastric and intestinal digestive solutions used is described in the United States Pharmacopoeia (1989). The exposure of mEPSPS to SGF and SIF was conducted over a series of timed incubations at 37°C. The products of the digestion were analysed using gel electrophoresis and Western blots.

The results of these experiments demonstrated that the mEPSPS protein was no longer detectable after 15 seconds in the gastric system and within one minute in the intestinal system. Moreover, the results of these simulated digestion experiments showed no evidence for the presence of stable peptide fragments larger than 2 kDa. These results provide evidence that the mEPSPS protein is readily digested in the mammalian digestive tract.

**Conclusion**

The family of EPSPS enzymes from various plant and microbial food sources are currently part of the human diet and have been consumed over a long period without any health concerns. The data and analyses on the potential for allergenicity of the mEPSPS protein support the conclusions that the protein is not derived from an allergenic food source, does not exhibit sequence similarity with known allergens, and does not exhibit the biochemical characteristics of known protein allergens. Furthermore, the protein is present in relatively low abundance in the grain and demonstrates digestive lability in conditions that mimic human digestion. These results strongly support the conclusion that corn line GA21 expressing mEPSPS does not pose any greater risk than conventional corn, with respect to potential allergenicity.

## NUTRITIONAL ISSUES

### Compositional analysis

Studies evaluated:

K.M. Magin, B.E. Ledesma, G.J. Rogan, P.R. Sanders and R.S. Sidhu, 1998. Expression and Compositional Analyses of the Roundup Ready® Corn Line GA21 Produced in 1996 Field Trials. Performing Laboratories: Monsanto Life Sciences Company and Covance Laboratories Inc. Report No. MSL-15196, Study 96-01-46-01, CHW Project 6103-199

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 untransformed) 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.

Transformed corn line GA21 and its non-transgenic control were grown in 1996 at five field locations in the United States, predominantly in the major corn growing belt. The control plants were the population of non-transgenic negative segregants (that is, plants lacking the mEPSPS gene addition) present in untreated plots of transgenic GA21 corn. The non-transgenic negative segregants were identified using the polymerase chain reaction (PCR) technique. The plants were individually self-pollinated, and the harvested forage and grain analysed in order to determine the compositional profile of the transgenic corn in comparison with that of conventional corn varieties grown commercially.

*Sample collection and preparation*

At the outset of the compositional studies, forage was defined as the entire plant minus the roots collected at the soft dough stage. Four plants were collected at the glyphosate treated plots to provide the treated GA21 samples. Two positive and two negative segregant plants were collected from the untreated plots to provide the untreated GA21 and control samples respectively. Non-transgenic segregants were used as the negative control because these are the same genetic line (isogenic) as the transformed line GA21.

At normal commercial harvest (approximately 25% grain moisture), all self-pollinated ears from up to 12 plants were collected from glyphosate treated plots to provide treated GA21 grain samples. Similarly, all ears from up to 12 self-pollinated plants tagged as positive and negative segregants were collected from untreated plots to provide untreated GA21 and control grain samples, respectively.

*Analyses*

The components measured included proximates (protein, fat, ash, carbohydrates, moisture, acid detergent fibre and neutral detergent fibre), amino acid composition, fatty acids profile, calcium and phosphorus in the grain, and proximates, calcium and

phosphorus in the forage. References were provided for all methods used in the analyses. The majority of methods were derived from standard methodology in *Official Methods of Analysis,* Association of Official Analytical Chemists and are validated AOAC International Methods, or American Oil Chemists Society (AOCS) Methods.

*Proximate, calcium and phosphorus analyses*

The corn kernal has been extensively studied for its composition, nutritional properties and feeding value, both as an important stock feed and as human food. It contains approximately 72% starch on a dry basis, is low in fibre, and relatively low in protein content (approximately 4%-20%). Like other cereal grains, corn is very low in calcium, and low in other minerals including phosphorus, potassium and magnesium. The oil in corn is highly polyunsaturated and rich in linoleic acid (2.9% of the whole corn on a dry basis).

The values for the proximate, calcium and phosphorus analyses of the corn grain are represented in the following summary Table of Results and are expressed as percent dry weight of the sample correcting for the measured moisture content. The mean value of each compositional parameter was calculated from measurements for each sample from each of the five test sites.

**Table 1 Results for the proximate and mineral analysis of corn grain**

| Component | Control (Untreated) Mean (%) | Untreated Line GA21 Mean (%) | Treated Line GA21 Mean (%) | Literature (Range %) |
|---|---|---|---|---|
| **Protein** | 10.1 | 10.0 | 9.9 | 6.0-12.0 |
| **Total fat** | 3.6 | 3.5 | 3.5 | 3.1-5.7 |
| **Ash** | 1.3 | 1.3 | 1.3 | 1.1-3.9 |
| **ADF** | 3.7 | 3.7 | 3.9 | 3.3-4.3 |
| **NDF** | 11.7 | 10.8 | 11.4 | 8.3-11.9 |
| **Carbohydrates** | 85.1 | 85.2 | 85.2 | |
| **Calcium** | 0.003 | 0.003 | 0.003 | 0.001-0.01 |
| **Phosphorus** | 0.3 | 0.3 | 0.29 | 0.26-0.75 |
| **Moisture** | 14.4 | 14.2 | 14.6 | 7-23 |

The results of the proximate analyses demonstrate that there are no statistically significant differences in these components between grain from control and untreated or treated glyphosate-tolerant corn line GA21. In addition, the values obtained in the experiments were either within the range in published literature (Watson, 1987; Jugenheimer, 1976) or within previously reported ranges for non-transgenic corn varieties.

*Amino acid composition of corn line GA21*

Corn protein content, and its amino acid ratios, may vary widely due to genetic manipulation by traditional plant breeders and to a lesser degree by crop year, soil fertility, crop management (especially nitrogen fertilisation), and climatic conditions (Wright, 1987).

A modified version of an AOAC International method was used to determine the amino acid composition of the grain from corn line GA21. Eighteen individual amino acids were quantitated using an automated amino acid analyser. Both treated and untreated GA21 grain was compared with the control (PCR identified negative segregant) and with the literature values for commercially grown corn. The results of these analyses appear in the summary table below. The values are expressed as a percent of total amino acids and represent the least squares mean of five samples, one from each test site. The literature range is taken from Watson (1982) and represents values as a percent of total protein (10.1%). The amino acid components indicated with an asterisk (*) show a statistically significant difference in the mean value when compared to the control.

**Table 2 Results of Amino acid analyses of corn grain**

| Amino Acid | Control (Untreated) Mean (%) | Untreated Line GA21 Mean (%) | Treated Line GA21 Mean (%) | Literature Range (% )⊗ | Reported Range (%)δ |
|---|---|---|---|---|---|
| **Methionine** | 2.0 | 2.0 | 2.0 | 1.0-2.1 | 1.3-2.6 |
| **Cysteine** | 2.1 | 2.1 | 2.1 | 1.2-1.6 | 1.8-2.7 |
| **Lysine** | 3.1 | 3.0 | 2.8 * | 2.0-3.8 | 2.6-3.5 |
| **Tryptophan** | 0.6 | 0.6 | 0.6 | 0.5-1.2 | 0.4-1.0 |
| **Threonine** | 3.7 | 3.8 | 3.8 | 2.9-3.9 | 3.3-4.2 |
| **Isoleucine** | 3.6 | 3.6 | 3.5 * | 2.6-4.0 | 3.2-4.3 |
| **Histidine** | 2.8 | 2.8 | 2.8 | 2.0-2.8 | 2.8-3.3 |
| **Valine** | 4.6 | 4.6 | 4.5 * | 2.1-5.2 | 4.2-3.5 |
| **Leucine** | 12.9 | 13.1 | 13.2 | 7.8-15.2 | 12.6-15.8 |
| **Arginine** | 4.3 | 4.1 | 4.0 * | 2.9-5.9 | 3.6-5.0 |
| **Phenylalanine** | 5.2 | 5.1 | 5.1 | 2.9-5.7 | 5.0-6.1 |
| **Glycine** | 3.8 | 3.7 | 3.7 | 2.6-4.7 | 3.2-4.2 |
| **Alanine** | 7.6 | 7.6 | 7.7 | 6.4-9.9 | 7.3-8.8 |
| **Aspartic Acid** | 6.7 | 6.7 | 6.6 | 5.8-7.2 | 6.3-7.5 |
| **Glutamic Acid** | 19.1 | 19.3 | 19.4 | 12.4-19.6 | 19.5-22.8 |
| **Proline** | 8.7 | 8.7 | 8.8 | 6.6-10.3 | 8.7-10.1 |
| **Serine** | 5.3 | 5.3 * | 5.4 * | 4.2-5.5 | 4.9-6.0 |
| **Tyrosine** | 3.9 | 3.8 * | 4.0 | 2.9-4.7 | 3.7-4.3 |

⊗Values are percent of total protein (10.1% total protein).
δ Data from five nontransgenic corn lines evaluated in 1993-5, Monsanto field trials.

Statistically significant differences between control and untreated or treated GA21 grain were not observed for a majority of the amino acids tested. The results showed that the mean differences from control in grain for untreated GA21 was significantly different in two amino acids. Serine was increased by 1.2% and tyrosine was decreased by 3.4%.

The mean differences for treated GA21 grain compared with the control were statistically significant for five amino acids. The mean values of four amino acids were lower than the control (lysine –8.6%, arginine –8.2%, valine –3.6% and isoleucine –2.6%). One amino acid, serine, was 2.4% greater in the GA21 line than in the control line. However, inspection of the raw data indicates that these differences are not biologically relevant as all values were either within the ranges published in

the literature (Watson, 1982) or within previously reported ranges for non-transgenic corn varieties.

*Fatty acid composition of corn line GA21*

Treated and untreated GA21 grain was compared with respect to fatty acid composition to non-transgenic control samples. Nine different fatty acid types were analysed and the results indicated no statistically significant differences between the values recorded for control and either treated or untreated GA21 grain for any fatty acid component, with one exception. A minor difference was observed in stearic acid content between the control (mean of 1.9% of total fatty acid) and the treated GA21 line (mean of 1.8% of total fatty acid). The results of the fatty acid composition analysis are presented in the following Table of Results:

**Table 3 Results of the fatty acids analysis of corn grain**

| Fatty Acid Mean (%) | Control Mean (%) | Untreated Line GA21 Mean (%) | Treated Line GA21 Mean (%) | Literature Range* (%) | Reported Range** (%) |
|---|---|---|---|---|---|
| **Arachidic (20:0)** | 0.4 | 0.4 | 0.4 | 0.1-2 | 0.3-0.5 |
| **Behenic (22:0)** | 0.2 | 0.2 | 0.2 | | 0.1-0.3 |
| **Eicosenoic (20:1)** | 0.3 | 0.3 | 0.3 | | 0.2-0.3 |
| **Linoleic (18:2)** | 58.7 | 58.6 | 59.1 | 35-70 | 55.9-66.1 |
| **Oleic (18:1)** | 27.4 | 27.5 | 27.1 | 20-46 | 20.6-27.5 |
| **Palmitic (16:0)** | 9.9 | 9.9 | 9.9 | 7-19 | 9.9-12.0 |
| **Palmitoleic (16:1)** | 0.2 | 0.2 | 0.2 | | |
| **Stearic (18:0)** | 1.9 | 1.9 | 1.8 | 1-3 | 1.4-2.2 |
| **Linolenic (18:3)** | 1.1 | 1.1 | 1.1 | 0.8-2 | 0.8-1.1 |

* Watson, 1982
** Data from five nontransgenic corn lines evaluated in 1993-5, Monsanto field trials.

Corn oil is an excellent source of polyunsaturated fatty acids, with a high level of the essential fatty acid linoleic acid (18:2). In addition, it has naturally low levels of the saturated fatty acids, palmitic acid (16:0, 11%) and stearic acid (18:0, 2%). It is known also that corn oil from cooler regions has a higher proportion of unsaturated fatty acids than corn oil from warmer areas, which appears to be an adaptation to climatic conditions. However, genotype has a greater influence on fatty acid composition than any environmental factor. The biochemical variability for fatty acid composition among corn genotypes is known to cover a broad range. Examination of the raw data therefore indicates that the minor observed difference in stearic acid levels in the transgenic corn is neither biologically relevant, since the value was

within the ranges published in the literature (Watson, 1982), nor is it an issue for food safety because of broad natural variation.

*Conclusions from compositional analyses*

Comprehensive data from a range of compositional analyses conducted on grain from both untreated and treated corn line GA21 and the non-transgenic control were presented for assessment. The compositional components measured included proximates (protein, fat, ash, carbohydrates, moisture, acid detergent fibre and neutral detergent fibre), amino acid composition, fatty acids profile, calcium and phosphorus. In addition, data were provided on proximates, calcium and phosphorus in the forage, but as this portion of the plant is not for human consumption, the data were not considered in the assessment process.

The results of the compositional data do not indicate that there are any biologically significant differences between glyphosate-tolerant corn line GA21, either untreated or following treatment with glyphosate, and the non-transgenic control in any of the parameters measured. Some minor statistically significant differences were observed in the amino acid composition of the treated GA21 grain in comparison with the control. The differences were observed for arginine, isoleucine, lysine, valine and serine, but were not considered to be of either biological relevance for commercially grown corn varieties nor of significance in terms of food safety.

Similarly, a minor statistical difference observed in the fatty acid profile specifically in the level of stearic acid in the treated GA21 line compared to the control was not considered to be significant as the value was within the known reported range for commercial corn varieties and is not of concern in terms of food safety.

**Levels of anti-nutrients**

Corn contains insignificant levels of anti-nutrient compounds. The levels of trypsin inhibitor in particular are known to be very low (Melville *et al*., 1972; Halim *et al*., 1973) and lectins, carbohydrate binding proteins with haemagglutination activity, have been found at low levels in the endosperm and germ (Newberg and Concon, 1985).

The content of trypsin and chymotrypsin inhibitors is traditionally determined by enzymatic methods, but these methods are very dependent on the concentration of protein, non-protein inhibitors and other factors.

**Ability to support typical growth and well being**

In assessing the safety of a genetically modified food, 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 GA21, animal feeding studies using the whole food have not been conducted. The nutritional profile of corn line GA21 was determined by compositional analyses of the major components of the kernel and these were found to be comparable to the conventional control lines. Further studies on the specific protein sequence found that no significant similarity to known allergens or toxins exists. An acute oral toxicity study in mice using variable amounts of mEPSPS as a single dose, found no evidence to indicate that the protein produces toxic effects in animals. In addition, the mEPSPS protein has been shown to be rapidly degraded in model digestive systems. Finally, the level of dietary exposure to the new protein has been estimated to be very low. The major human food uses for corn are the extensively processed starch and oil fractions yielding high fructose corn syrup and corn oil, neither containing protein. Human exposure to the new protein from whole grain corn in the diet is also considered to be very low due both to its low abundance in the protein fraction of the grain and to the proportionately low percentage of protein in the kernal, compared with the major starch component.

In view of the safety data available for this food and the technical features of the genetic modification, it is considered that, for this application, additional studies on wholesomeness were not essential to the safety assessment process. The donor of the mEPSPS gene and the recipient organism are the same plant species, namely corn (*Zea mays* L.). The modified gene was generated *in vitro,* has been entirely sequenced, and the properties of the encoded protein have been biochemically characterised.

In summary, the data and information available on the genetic change, together with the composition data, provide a sound basis for consideration of the safety of this food without requiring feeding studies in laboratory animals.

## OTHER MATTERS

### Dietary Exposure

The applicant has provided an estimate of the human dietary exposure to mEPSPS protein. The analysis provides a worst-case estimate of exposure to mEPSPS and assumes no breakdown of the protein during preparation of various corn fractions by wet or dry milling procedures.

Ingestion of products derived from corn line GA21 was estimated to provide approximately 0.02 mg/kg bw/day of mEPSPS for general consumers in the US, where corn products are normally a high dietary component. A concentration of mEPSPS of 0.001% (fresh weight basis) was used in this analysis which was approximately 25% higher than the highest value found in corn grain from 1996 US field trials conducted at five separate locations. Levels of the wildtype corn EPSPS protein in the non-transgenic control line were below the detection limit (0.00015%) of the ELISA method used for analysis. These results demonstrate that, even with a

relatively high pattern of usage of corn products, human dietary exposure to mEPSPS is expected to be very low.

**ACKNOWLEDGEMENTS**

ANZFA gratefully acknowledges Professor Richard Head of CSIRO Health Sciences and Nutrition, Adelaide, and Professor Geoff Fincher of the Department of Plant Science at the University of Adelaide, for their expert comments on this safety assessment report.

## REFERENCES


Australia New Zealand Food Authority (ANZFA), 1999. Guidelines for the safety assessment of foods to be included in Standard A18 - Food Produced Using Gene Technology.

Della-Cioppa,G., Bauer, S.C., Klein, B.K., Shah, D.M., Fraley, R.T. and Kishore G.M., 1986. Translocation of the precursor of 5-enolpyruvylshikimate-3-phosphate synthase into chloroplasts of higher plants *in vitro*. Proc.Natl.Acad.Sci. 83, 6873-6877.

Doolittle, R.F. 1990. Searching through sequence databases. Methods in Enzymology 183, 99-110.

Frick, O.L., 1995. The potential for allergenicity in transgenic foods. In *Genetically Modified Foods: Safety Aspects*. Editors K.H. Engel, G.R. Takeoka and R. Teranishi. American Chemical Society, Washington DC.

Halim, A.H., Wassom, C.E. and Mitchell, H.L., 1973. Trypsin inhibitor in corn (*Zea mays* L.) as influenced by genotype and moisture stress. Crop Sci. 13, 405-406.

Jugenheimer, R.W., 1976. Corn Improvement, Seed Production and Uses. John Wiley and Sons, Inc. New York, New York, USA.

Lebrun, M., Leroux, B. and Sailland, A., 1996. Chimeric gene for the transformation of plants. U.S. patent No. 5,510,471.

McElroy, D., Zhang, W., Cao, J. and Wu, R., 1990. Isolation of an Efficient Actin Promoter for Use in Rice Transformation. The Plant Cell, 2, 163-171.

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. Critical Reviews in Food Science and Nutrition 36(S): S165-S186.

Mousedale, D.M. and Coggins, J.R. 1984. Purification of properties of 5-enolpyruvylshikimate-3-phosphate synthase from seedling of *Pisum sativum*. L. Planta 160, 78-83.

Padgette, S.R., Biest Re, D., Gasser, C.S., Eichholtz, D.A., Frazier, R.B., Hironaka, C.M., Levine, E.B., Shah, D.M., Fraley R.T. and Kishore G.M., 1991. Site–directed Mutagenesis of a Conserved Region of the 5-Enolpyruvylshikimate-3-phosphate Synthase Active Site. J. Biol. Chem. 266, 33, 22364-22369.

San Miguel, P., Tikhonov, A., Jin,Y-K., Motchoulskaia, N., Zakharov, D., Melake-Berhan, A., Springer, P.S., Edwards, K.J., Lee, M., Avramovoa, Z. and Bennetzen, J.L., 1996. Nested retrotransposons in the intergenic regions of the maize genome. Science, 274, 765-768.

Schultz, A., Kruper, A. and Amrhein, N., 1985. Differential sensitivity of bacterial 5-enolpyruvylshikimate-3-phosphate synthases to the herbicide glyphosate. FEMS Microbiology Letters, 28, 297-301.

Wright, K.N., 1987. Nutritional properties and feeding value of corn and its by-products, pp 447-478. *In* Corn: Chemistry and Technology. S.A.Watson and P.E. Ramsted (Eds.), American Association of Cereal Chemists, Inc. St. Paul, Minnesota, USA.

Watson, S.A., 1987. Structure and Composition, pp. 53-82. *In* Corn: Chemistry and Technology. S.A. Watson and P.E. Ramstad, (Eds.), American Association of Cereal Chemists, Inc. St. Paul, Minnesota, USA.

Watson, S.A., 1982. Corn: Amazing Maize: General Properties. In CRC Handbook of Processing and Utilization in Agriculture, Volume II: Part1 Plant Products. I.A.Wolff (ed.) CRC Press Inc., Florida, USA.

World Health Organisation (WHO), 1995. Application of the Principles of Substantial Equivalence to the Safety Evaluation of Foods or Food Components from Plants Derived by Modern Biotechnology. Report of a WHO workshop.