12 December 2001
06/02

DRAFT ASSESSMENT
(Full Assessment - s.15)

APPLICATION A416

Food derived from
Glyphosate-tolerant Corn Line NK603

DEADLINE FOR PUBLIC SUBMISSIONS to the Authority in relation to this matter:
23 January 2002 (See 'Invitation for Public Submissions' for details)

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EXECUTIVE SUMMARY

Background

An application was received from Monsanto Australia Limited on 29 May 2000 seeking approval for food derived from genetically modified (GM) corn line NK603 under Standard A18 – Food Produced Using Gene Technology, Volume 1 of the Australian *Food Standards Code* (Standard 1.5.2 in Volume 2). The line is modified for tolerance to the herbicide glyphosate, known commercially as Roundup®. This report describes the scientific assessment of the application.

Issues addressed during assessment

i. Safety Evaluation

Nature of the genetic modification

In this application, the glyphosate-tolerance trait has been introduced into corn plants by the addition of a bacterial gene encoding the EPSPS protein, a key enzyme in the biosynthesis of aromatic amino acids in plants and microbes. The mode of action of glyphosate is to bind to the plant EPSPS protein, thereby impairing its normal enzyme activity, subsequently resulting in plant cell death. The bacterial form of the enzyme (denoted as CP4 EPSPS) has a lower affinity for glyphosate, so that when present in plant cells, the activity of the introduced enzyme replaces the sensitive plant EPSPS enzyme. The result is that the engineered plant is able to function in the presence of the herbicide.

Line NK603 contains two linked copies of the CP4 EPSPS gene, each with separate regulatory sequences. One copy is expressed from the rice actin promoter and intron while the second is expressed from the enhanced cauliflower mosaic virus promoter, which have both been shown to direct constitutive protein expression in corn. Additional regulatory sequences in common include an optimised chloroplast transit peptide sequence, to direct translocation of the CP4 EPSPS protein to chloroplasts where the protein is functionally active, and a NOS 3' untranslated region providing the appropriate eukaryotic polyadenylation signal. Because a purified segment of DNA was used in the transformation, no extraneous bacterial genes, including laboratory marker genes, were transferred.

General safety 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. The bacterial gene used in corn line NK603 is derived from a common soil bacterium, *Agrobacterium* sp. strain CP4 which is not pathogenic. Comprehensive analytical data on the modified corn is available. There is only one new protein, namely the CP4 EPSPS enzyme, produced by the genetic modification. This new protein is present in corn grain, however the family of EPSPS proteins are ubiquitous in plant and microbial food sources that are already part of human diets.

Toxicology issues

The chemical similarity, and functional identity, of the CP4 EPSPS protein to other EPSPS proteins already consumed as part of the human diet provide some evidence that there is no inherent toxicity associated with the introduced protein. This was supported by the results of an acute toxicity study in mice, where animals were given purified CP4 EPSPS protein at single dose levels up to 400 mg/kg. There were no clinical signs of toxicity and animals continued to grow normally for the duration of the 9 day study.

Similarly, there is no evidence to indicate that food derived from corn line NK603 would be more likely to cause allergies than food derived from the non-transformed counterpart. The CP4 EPSPS 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. In addition, there is no possibility for the transfer of marker genes to cells in the human digestive tract from the consumption of food products derived from NK603 corn as the transformation was achieved using a purified DNA segment that did not include extraneous genetic material or antibiotic resistance marker genes.

Nutritional issues

All parts of the grain may be used to produce food fractions including corn oil, flour, starch and sugars, particularly high fructose corn syrup. The results of extensive compositional analyses on glyphosate-treated plants grown at multiple locations demonstrate that the levels of the important components in NK603 corn grain (protein, total fat, carbohydrate, ash, fibre, fatty acids, amino acids, minerals and moisture) are not different from the non-transformed parental line. In addition, analyses for Vitamin E, phytic acid and trypsin inhibitor confirmed that the modification has not resulted in any variation to these minor components.

Statistical analysis of the results for fatty acids and amino acids showed that some minor differences between the transformed line and non-transformed control line occurred at one or two of the trial sites. However, the nature of the differences was not consistent across all sites in the two major studies and therefore the differences were considered to reflect random variation that is characteristic of large-scale plant analyses. Moreover, all compositional results from the transformed line were well within the ranges observed for commercial non-transformed lines for each of the parameters investigated.

Corn line NK603 was also shown to be equivalent to its non-transformed counterpart in the ability to support typical growth and well being when included in the diet of rapidly growing broiler chickens.

Conclusion of the safety assessment

EPSPS enzymes from various plant and microbial food sources have been part of the protein component of the human diet over thousands of years, and are not associated with any known health concerns. The safety of food derived from glyphosate-tolerant corn line NK603 is based on:

a) a thorough understanding of the genetic modification and identification of the new gene product;

b) characteristics of the CP4 EPSPS protein in relation to its potential toxicity or allergenicity;
c) compositional analysis of the modified corn line compared to traditional corn lines.

The conclusion from this assessment is that, on the basis of the available evidence, glyphosate-tolerant corn line NK603 is compositionally equivalent to unmodified corn varieties, and is therefore suitable for human food use with respect to its safety, nutritional properties and wholesomeness.

ii. Labelling

Under the current Standard A18, which remains in effect until 7 December 2001, food derived from glyphosate-tolerant corn line NK603 would not require labelling as it is regarded as '*substantially equivalent'* to food derived from the non-genetically modified counterpart.

When the amended Standard (A18 in Volume 1, 1.5.2 in Volume 2 of the *Food Standards Code*) comes into effect on 7 December 2001, food products derived from NK603 corn will require labelling if novel DNA and/or protein is present in the final food.

iii. Public Submissions

Six submissions were received in response to the public notification of this application, of which one was supportive. Those opposing the application did so primarily on the basis that they perceive foods produced from GM crops to be unsafe, irrespective of the specific nature of the modification. The food safety concerns raised in submissions have been addressed by the draft safety assessment report.

iv. Review by external panel

It was not considered necessary to seek comments on the draft safety assessment from members of the external panel of experts, as this application deals with the insertion of a bacterial gene encoding the CP4 EPSPS protein which confers tolerance to glyphosate. The same gene was used in other food commodities such as glyphosate-tolerant soybeans, cotton, canola and sugarbeet that have already been assessed by ANZFA. In addition, several other genetically modified corn lines have undergone a safety assessment. The Draft Assessment Report for each of these previously assessed foods has been referred for independent external review and the conclusions have subsequently been endorsed by the reviewers. In addition, with the exception of glyphosate-tolerant sugarbeet which has undergone recent assessment, all of the foods derived from commodities modified with the CP4 EPSPS gene have been approved by the Ministerial Council.

Conclusions

On the basis of the data submitted with the application, evidence obtained from the scientific literature and from information obtained from public submissions, it is concluded that:

- The introduced genes in NK603 corn are not considered to produce any additional public health and safety risk;

- Food derived from NK603 corn is as safe and wholesome as food from other commercially available corn varieties;

- From 7 December 2001, food products containing NK603 corn will require labelling if novel DNA and/or protein is present in the final food; and

- The proposed amendment to the *Food Standards Code* is consistent with the section 10 objectives of the *Australia New Zealand Food Authority Act* 1991 and the regulatory impact assessment.

ANZFA now seeks public comment on the proposed amendment in accordance with the procedures described in Section 16 of the *Australia New Zealand Food Authority Act* 1991.

FOOD STANDARDS-SETTING IN AUSTRALIA AND NEW ZEALAND

The Governments of Australia and New Zealand entered an Agreement in December 1995 establishing a system for the development of joint food standards. On 24 November 2000, Health Ministers in the Australia New Zealand Food Standards Council (ANZFSC) agreed to adopt the new *Australian New Zealand Food Standards Code*. The new Code was gazetted on 20 December 2000 in both Australia and New Zealand as an alternate to existing food regulations until December 2002 when it will become the sole food code for both countries. It aims to reduce the prescription of existing food regulations in both countries and lead to greater industry innovation, competition and trade.

Until the joint *Australia New Zealand Food Standards Code* is finalised the following arrangements for the two countries apply:

- **Food imported into New Zealand other than from Australia** must comply with either Volume 1 (known as Australian *Food Standards Code)* or Volume 2 (known as the joint *Australia New Zealand Food Standards Code)* of the Australian *Food Standards Code,* as gazetted in New Zealand, or the New Zealand *Food Regulations 1984,* but not a combination thereof. However, in all cases maximum residue limits for agricultural and veterinary chemicals must comply solely with those limits specified in the New Zealand *(Maximum Residue Limits of Agricultural Compounds) Mandatory Food Standard 1999.*

- **Food imported into Australia other than from New Zealand** must comply solely with Volume 1 (known as Australian *Food Standards Code)* or Volume 2 (known as the joint *Australia New Zealand Food Standards Code)* of the Australian *Food Standards Code,* but not a combination of the two.

- **Food imported into New Zealand from Australia** must comply with either Volume 1 (known as Australian *Food Standards Code)* or Volume 2 (known as *Australia New Zealand Food Standards Code)* of the Australian *Food Standards Code* as gazetted in New Zealand, but not a combination thereof. Certain foods listed in Standard T1 in Volume 1 may be manufactured in Australia to equivalent provisions in the New Zealand *Food Regulations 1984.*

- **Food imported into Australia from New Zealand** must comply with Volume 1 (known as Australian *Food Standards Code)* or Volume 2 (known as *Australia New Zealand Food Standards Code)* of the Australian *Food Standards Code,* but not a combination of the two. However, under the provisions of the Trans-Tasman Mutual Recognition Arrangement, food may **also** be imported into Australia from New Zealand provided it complies with the New Zealand *Food Regulations 1984.*

- **Food manufactured in Australia and sold in Australia** must comply with Volume 1 (known as Australian *Food Standards Code)* or Volume 2 (known as *Australia New Zealand Food Standards Code)* of the Australian *Food Standards Code* but not a combination of the two. Certain foods listed in Standard T1 in Volume 1 may be manufactured in Australia to equivalent provisions in the New Zealand *Food Regulations 1984.*

In addition to the above, all food sold in New Zealand must comply with the New Zealand *Fair Trading Act 1986* and all food sold in Australia must comply with the Australian *Trade Practices Act 1974*, and the respective Australian State and Territory *Fair Trading Acts.*

Any person or organisation may apply to ANZFA to have the *Food Standards Code* amended. In addition, ANZFA may develop proposals to amend the Australian *Food Standards Code* or to develop joint Australia New Zealand food standards. ANZFA can provide advice on the requirements for applications to amend the *Food Standards Code*.

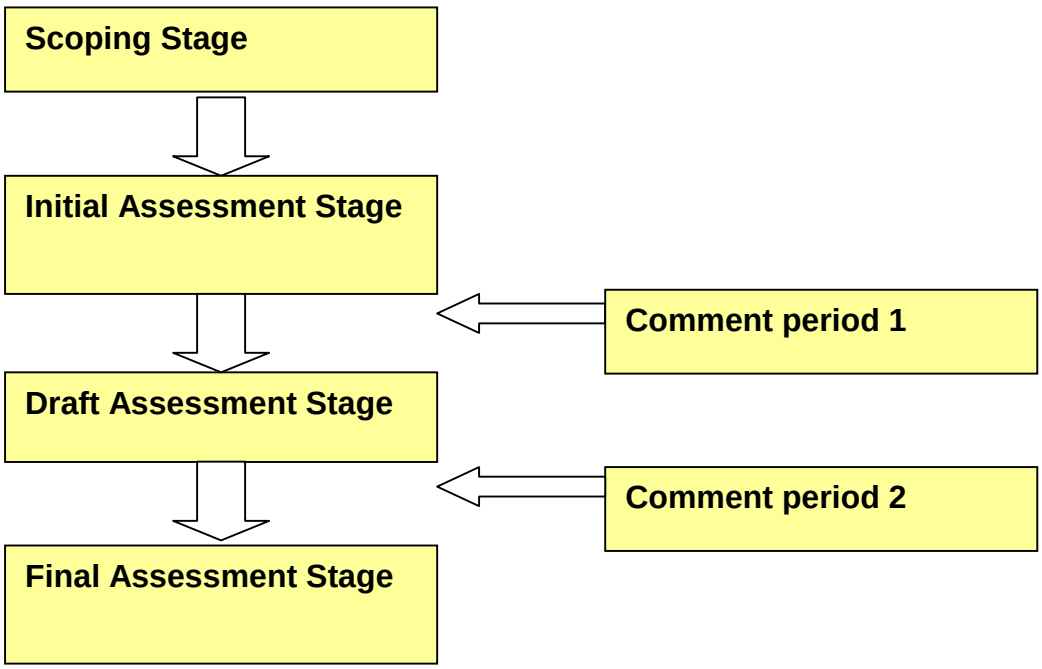
INVITATION FOR PUBLIC SUBMISSIONS

The process for amending the *Australia New Zealand Food Standards Code* (the Code) is prescribed in the ANZFA Act 1991. Open and transparent consultation with interested parties is a key element in the process involved in amending or varying the Code.

Any individual or organization may make an 'application' to the Australia New Zealand Food Authority (the Authority) seeking to change the Code. The Authority itself, may also seek to change the Code by raising a 'proposal'. In the case of both applications and proposals there are usually two opportunities for interested parties to comment on proposed changes to the Code during the assessment process. This process varies for matters that are urgent or minor in nature.

Following the initial assessment of an application or proposal the Authority may decide to accept the matter and seek the views of interested parties. If accepted, the Authority then undertakes a draft assessment including, preparing a draft standard or draft variation to a standard (and supporting draft regulatory impact statement). If a draft standard or draft variation is prepared, it is then circulated to interested parties, including those from whom submissions were received, with a further invitation to make written submissions on the draft. Any such submissions will then be taken into consideration during the final assessment, which the Authority will hold to consider the draft standard or draft variation to a standard.

Comment opportunities in the usual assessment process to change the Australia New Zealand Food Standards Code
(Note: this process may vary for matters that are urgent or minor)



Content of Submissions

Written submissions containing technical or other relevant information which will assist ANZFA in undertaking an assessment on matters relevant to the application, including consideration of its regulatory impact, are invited from interested individuals and organizations. Information providing details of potential costs and benefits of the proposed change to the Code from stakeholders is highly desirable. Claims made in submissions should be supported wherever possible by referencing or including relevant; studies, research findings, trials, surveys etc. Technical information presented should be in sufficient detail to allow independent scientific assessment.

Submissions may provide more general comment and opinion on the issue although those framing their submissions should bear in mind ANZFA's regulatory role specifically relates to food supplied for human consumption in Australia and New Zealand. The ANZFA Act 1991 sets out the objectives of the Authority in developing food regulatory measures and variations of food regulatory measures as:

(a) the protection of public health and safety; and
(b) the provision of adequate information relating to food to enable consumers to make informed choices; and
(c) the prevention of misleading or deceptive conduct.

In developing food regulatory measures and variations of food regulatory measures
The Authority must also have regard to the following:

(a) the need for standards to be based on risk analysis using the best available scientific evidence;
(b) the promotion consistency between domestic and international food standards;
(c) the desirability of an efficient and internationally competitive food industry;
(d) the promotion of fair trading in food.

Submissions addressing the issues in the context of the objectives of the Authority as set out in the *ANZFA Act 1991* will be more effective in supporting their case.
Written submissions containing technical or other relevant information which will assist the Authority in undertaking a final assessment on matters relevant to the application, including consideration of its regulatory impact, are invited from interested individuals and organisations. Technical information presented should be in sufficient detail to allow independent scientific assessment.

Submissions providing more general comment and opinion are also invited. The Authority's policy on the management of submissions is available from the Standards Liaison Officer upon request.

Following its draft assessment of the application the Authority may prepare a draft standard or draft variation to a standard (and supporting draft regulatory impact statement), or decide to reject the application/proposal. If a draft standard or draft variation is prepared, it is then circulated to interested parties, including those from whom submissions were received, with a further invitation to make written submissions on the draft. Any such submissions will then be

taken into consideration during the inquiry, which the Authority will hold to consider the draft standard or draft variation to a standard.

Transparency
The processes of ANZFA are open to public scrutiny, and any submissions will ordinarily be placed on the public register of ANZFA and made available for inspection. If you wish any confidential information contained in a submission to remain confidential to ANZFA, you should clearly identify the sensitive information and provide justification for treating it in confidence. The *Australia New Zealand Food Authority Act 1991* requires ANZFA to treat in confidence trade secrets relating to food and any other information relating to food, the commercial value of which would be or could reasonable be expected to be destroyed or diminished by disclosure.

Contact details for submitters are recorded so that the Authority can continue to keep them informed about progress of the application or proposal.

Deadlines
The deadlines for submissions are clearly indicated in the advertisements calling for comment and in the relevant Assessment Reports. While the Authority often provides comment periods of around 6 weeks, the periods allowed for comment may vary and may be limited to ensure critical deadlines for projects can be met. Unless the Project Manager has given specific consent for an extension, the Authority cannot guarantee that submissions received after the published closing date will be considered.

Delivery of Submissions
Submissions must be made in writing and should be clearly marked with the word 'Submission' and quote the correct project number and title. Submissions may be sent by mail, fax or email to one of the following addresses:

Australia New Zealand Food Authority
PO Box 7186
Canberra BC ACT 2610
AUSTRALIA
Tel (02) 6271 2258
Fax (02) 6271 2278
email: slo@anzfa.gov.au

Australia New Zealand Food Authority
PO Box 10559
The Terrace WELLINGTON 6036
NEW ZEALAND
Tel (04) 473 9942
Fax (04) 473 9855
email: anzfa.nz@anzfa.gov.au

Submissions should be received by the Authority by: 23 JANUARY 2002

Submissions may also be sent electronically through the submission form on the ANZFA website www.anzfa.gov.au. Electronic submissions should also include the full contact details of the person making the submission on the main body of the submission so that the contact details are not separated.

Further Information
Further information on this and other matters should be addressed to the Standards Liaison Officer at the Australia New Zealand Food Authority at one of the above addresses.

Assessment reports are available for viewing and downloading from the ANZFA website or alternatively paper copies of reports can be requested from the Authorities Information Officer at info@anzfa.gov.au.

BACKGROUND TO THE APPLICATION

ANZFA received an application from Monsanto Australia Ltd on 29 May 2000 seeking amendment to the Australian *Food Standards Code* to include food derived from glyphosate-tolerant corn line NK603 in the Table to clause 2 of Standard A18 – Food Produced Using Gene Technology (Standard 1.5.2. in the joint Australia New Zealand Food Standards Code).

Corn line NK603 has been modified for tolerance to the broad spectrum herbicide glyphosate, the active ingredient in the proprietary herbicide with the commercial name Roundup®. The bacterial gene used to confer tolerance to glyphosate in this application is the same gene used in certain genetically modified varieties of soybean, canola, sugar beet, corn and cotton. With the exception of glyphosate-tolerant sugar beet, which has been assessed by ANZFA but not yet approved, foods derived from these modified crop lines have already undergone a safety assessment and have been approved[1] in Australia and New Zealand under Standard A18/1.5.2.

Glyphosate directly affects the shikimate biosynthetic pathway in plants. 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. The herbicide thus results in a breakdown of the synthesis of essential aromatic amino acids in the cell, ultimately leading to the death of that cell.

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). Tolerance to glyphosate in plants can therefore be achieved by introducing a bacterial 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 NK603, the glyphosate-tolerance trait is generated in the plants through the addition of a bacterial EPSPS gene derived from a common soil bacterium, *Agrobacterium* sp. strain CP4 (CP4 EPSPS). The enzyme produced from the CP4 EPSPS gene has a reduced affinity for the herbicide compared with the corn enzyme, and thus confers glyphosate tolerance to the whole plant.

Corn is used predominantly as an ingredient in the manufacture of breakfast cereals, baking products, extruded confectionery and corn chips. Maize starch is used extensively by the food industry for the manufacture of many processed foods including dessert mixes and canned foods. Corn oil may also be used in various edible oils and margarine.

Despite the diverse uses of corn products in many foods, corn is a relatively minor crop in both Australia and New Zealand with a declining area planted over the last decade. Australian and New Zealand commercial corn production may be supplemented therefore by imported product, such as high-fructose corn syrup and maize starch, to meet manufacturing demand.

[1] The *Food Standards Code*, refer to the Table to clause 2 of Standard A18-Food Produced Using Gene Technology (Volume1), or Standard 1.5.2 (Volume 2).

Glyphosate-tolerant corn line NK603 is currently not approved for commercial planting in either Australia or New Zealand and is not one of the 20 applications received by ANZFA prior to April 30, 1999. The clause 2A transitional arrangements do not apply to corn line NK603 and therefore it does not have an interim permission to be present currently in food in Australia or New Zealand.

Glyphosate-tolerant corn line NK603 is currently planted commercially and consumed as food in the USA. It is undergoing the assessment process for feed and food use in Canada, Japan, the European Union, and Switzerland.

PUBLIC CONSULTATION

ANZFA completed an Initial Assessment (currently referred to as the Preliminary Assessment) upon receipt of the application and invited submissions from the public between 29 November 2000 and 24 January 2001. A total of 6 submissions was subsequently received and a summary of these is included in this report at Attachment 6.

NOTIFICATION OF THE WORLD TRADE ORGANIZATION

During the ANZFA assessment process, comments are also sought internationally from other Members of the World Trade Organization (WTO). As Members of the WTO, Australia and New Zealand are signatories to the agreements on the Application of Sanitary and Phytosanitary Measures (SPS Agreement) and on Technical Barriers to Trade (TBT Agreements). Further details on the WTO are included in Attachment 5. In some circumstances, Australia and New Zealand have an obligation to notify the WTO of changes to food standards to enable other member countries of the WTO to make comment.

As there is significant international interest in the safety of genetically modified foods, and the proposed changes to Standard A18/1.5.2 will have a liberalising effect on trade, the outcome of this application will therefore be notified to the WTO.

ISSUES ADDRESSED DURING ASSESSMENT

1. Safety assessment

The safety assessment of food derived from corn line NK603 has been completed according to the safety assessment guidelines prepared by ANZFA[2]. The evaluation considered the following issues: (1) the nature of the genetic modification; (2) general safety issues such as novel protein expression and the potential for transfer of novel genetic material to bacteria in the human digestive tract; (3) toxicological issues; and (4) nutritional issues. The completed safety assessment report is included in this document at **Attachment 3**.

On the basis of the combined available information, ANZFA concluded that food derived from NK603 corn is as safe for human consumption as food derived from other commercial corn varieties.

[2] ANZFA (2001) Information for Applicants – Amending Standard A18/Standard 1.5.2 - Food Produced Using Gene Technology.

2. Labelling of foods derived from corn line NK603

On 28 July 2000 the Australia New Zealand Food Standards Council agreed to a revised standard which requires labelling of food where novel DNA and/or protein is present in the final food and also where the food has altered characteristics. The revised standard (A18 in the Australian *Food Standards Code*, 1.5.2 in the Australia New Zealand Food Standards Code) was gazetted on 7 December 2000 and will come into effect in both countries 12 months from the date of gazettal. Until the new labelling requirements take effect, the provisions in the current Standard A18/1.5.2 apply.

Under the new provisions, some food products derived from corn line NK603 will be likely to require labelling due to the presence of novel protein and/or novel DNA. Foods such as corn flour are produced from the meal and would be expected to contain corn proteins. Conversely, the refining processes required to produce corn oil are likely to exclude plant DNA and proteins. Similarly, high fructose corn syrup, a highly processed carbohydrate fraction, would not be expected to contain plant DNA or proteins. Ultimately, the requirement for labelling of foods derived from corn line NK603 will be determined by the particular corn fraction used and the degree of processing that occurs to achieve the final food.

3. Issues arising from public submissions

The Authority received 6 submissions in response to the invitation for the first round of public comment on this application. Most of the submissions provided comments on general issues relating to gene technology rather than on issues relating specifically to corn line NK603. Although a discussion of the general issues is included at Attachment 6, where further concerns have been expressed a more detailed response is presented below.

(i) Comments on behalf of the Safe Food Campaign (NZ)

The Safe Food Campaign strongly oppose the application on social and general safety grounds. The comments include concern that the spray residue in the corn is likely to increase as more herbicide is used.

Response

This is an issue that is frequently raised in submissions and a general response is included in Attachment 7 to this report at discussion point 14.

It is normal practice in primary production to use a range of different herbicides on conventional crop plantings, selecting appropriate weed treatment depending on the nature of the crop and the particular stage of plant development. The use of a GM herbicide tolerant crop generally results in an altered treatment regime for weeds in favour of the corresponding herbicide. In the case of glyphosate-tolerant crops, the use of glyphosate is possible throughout various stages of plant development due to the modification, whereas for traditional crops other herbicides are favoured at different times. Overall, the result of this altered spraying regime is a greater reliance on one broad spectrum herbicide with a concomitant reduction in the use of other herbicides to treat weed infestations.

Because of its low toxicity to humans and to the environment, glyphosate is used widely in agriculture. As reflected in Standard 1.4.2 Maximum Residue Limits (MRL, Australia only) of the *Food Standards Code* (Volume 2), its use is permitted in the production of a broad range of human foods including a variety of fruits, vegetables, nuts and cereal grains. The purpose of this standard is to set a level of residue that does not adversely affect human health while allowing good agricultural practice. The MRL applies to the food, irrespective of the commercial crop variety from which the food is derived (see Attachment 7, discussion point 14 for further details).

The Codex Alimentarius Commission, which is responsible for international food standards, recently concluded that separate MRLs should **not** be elaborated for GM and conventional crops (Codex Committee on Pesticide Residues, The Hague, The Netherlands, April 2001). In general, it was agreed that the existing MRLs were equally applicable to conventional and GM crops. The Committee advocated a case-by-case assessment in relation to the likely changes in the pattern of usage of a herbicide, or the requirement for residue chemical studies to be submitted as part of the safety assessment, where no previous MRL has been established for that food.

It is important to note that herbicide tolerance in plants occasionally occurs naturally, or may be enhanced in crops by conventional plant breeding. The relevant maximum residue limit also applies to the foods produced from these non-GM varieties, as it does to all other varieties including those that have been generated using recombinant DNA techniques.

4. Risk management

Under Standard A18 (and Standard 1.5.2 in the Australia New Zealand Food Standards Code), a GM food must undergo a safety assessment in accordance with ANZFA's safety assessment guidelines. The requirement for the food to be labelled must also be assessed in accordance with the labelling criteria specified in clause 4 of the amended standard. Labelling according to the original Standard A18 must be in accordance with the criteria specified in clause 2 and would be permitted until 7 December 2001. After this date, labelling is required to comply with Standard 1.5.2 of the Australia New Zealand Food Standards Code.

On the basis of the conclusions of the safety assessment report, together with a consideration of the issues raised in public submissions, it is proposed that the Table to clause 2 of Standard A18 be amended to include glyphosate-tolerant corn line NK603. The proposed amendment is provided in Attachment 1, including for the corresponding Standard 1.5.2 (Volume 2).

A public discussion paper on the safety assessment process for GM food[3] is widely available and may assist in addressing some of the concerns raised by the public. Other government and industry bodies, for example the Office of the Gene Technology Regulator (OGTR) in Australia and the Environmental Risk Management Authority (ERMA) in New Zealand, are also actively addressing broader issues concerning gene technology.

[3] ANZFA (2000) GM foods and the consumer: ANZFA's safety assessment process for genetically modified foods. ANZFA Occasional Paper Series No. 1.

5. Regulatory Impact Assessment

The advantages and disadvantages associated with the proposed amendment to Standard A18/1.5.2 have been analysed in a draft Regulatory Impact Assessment (Attachment 4). The benefits of the proposed amendment to approve glyphosate-tolerant corn line NK603 primarily accrue to primary production sectors of the food industry. On the assumption that corn line NK603 adds value to the pool of commercial varieties that are available to primary producers, there is a potential flow-on benefit to consumers by way of more favourable agronomic production costs.

CONCLUSIONS

ANZFA has conducted a comprehensive assessment of the application according to its revised *Guidelines for the safety assessment of foods to be included in Standard A18/Standard 1.5.2 – Food Produced Using Gene Technology*. These guidelines are based on internationally accepted principles for establishing the safety of foods derived from genetically modified organisms.

It is concluded that:

- the introduced genes in corn line NK603 are not considered to produce any additional public health and safety risk;

- based on the data submitted in the present application, glyphosate-tolerant corn line NK603 is equivalent to other commercial non-genetically modified corn varieties in terms of its food safety and nutritional adequacy.

ATTACHMENTS

1. Draft variation to the *Food Standards Code*
2. Draft Statement of Reasons
3. Draft safety assessment report
4. Draft regulatory impact assessment
5. World Trade Organisation Agreements
6. Summary of public comments
7. General issues raised in public comments

ATTACHMENT 1

DRAFT VARIATION TO THE *FOOD STANDARDS CODE*

A416 – FOOD DERIVED FROM GLYPHOSATE-TOLERANT CORN LINE NK603

To commence : On gazettal

The Food Standards Code is varied by:

[1] *inserting into Column 1 of the Table to clause 2 of Standard A18 of Volume 1* -

Food derived from glyphosate-tolerant corn line NK603

[2] *inserting into Column 1 of the Table to clause 2 of Standard 1.5.2 of Volume 2*-

Food derived from glyphosate-tolerant corn line NK603

ATTACHMENT 2

DRAFT STATEMENT OF REASONS

APPLICATION A416 – FOOD DERIVED FROM GLYPHOSATE-TOLERANT CORN LINE NK603

The Australia New Zealand Food Authority (ANZFA) has before it Application A416 (received on 29 May 2000) from Monsanto Australia Ltd, seeking approval for food derived from genetically modified (GM) corn line NK603 under Standard A18 – Food Produced Using Gene Technology, Volume 1 of the Australian *Food Standards Code* (Standard 1.5.2, Volume 2). The corn has been genetically modified for tolerance to glyphosate, the active ingredient of the broad spectrum herbicide known commercially as Roundup®. ANZFA has completed a Draft Assessment of the application and, in accordance with the conclusions of the assessment, has prepared draft variations to the *Food Standards Code*.

ANZFA recommends the adoption of the draft variations for the following reasons:

- the introduced genes in glyphosate-tolerant corn line NK603 are not considered to produce any additional public health and safety risk;

- on the basis of the data submitted in the present application, food derived from glyphosate-tolerant corn line NK603 is as safe and wholesome as food derived from other commercial varieties of corn;

- due to the presence of novel DNA or protein, some food fractions produced from corn line NK603 are likely to require labelling from 7 December 2001; and

- the proposed amendments to the *Food Standards Code* are consistent with the section 10 objectives of the *Australia New Zealand Food Authority Act 1991* and the regulatory impact assessment.

The commencement date of the draft variations will be the date of gazettal.

REGULATION IMPACT

ANZFA has undertaken a regulation impact assessment that also fulfils the requirement in New Zealand for an assessment of compliance costs. Consideration of the regulatory impact for foods produced using gene technology concludes that the benefits of permitting these foods primarily accrue to the primary production sectors of the food industry, particularly to corn producers, and to government. There is potentially a small flow-on benefit to consumers by way of reduced agronomic costs. These benefits are considered to outweigh the costs to government, consumers and industry, provided the safety assessment does not identify any public health and safety concerns.

WORLD TRADE ORGANIZATION (WTO) NOTIFICATION

Australia and New Zealand are members of the WTO and are bound as parties to WTO agreements. In Australia, an agreement developed by the Council of Australian Governments (COAG) requires States and Territories to be bound as parties to those WTO agreements to which the Commonwealth is a signatory. Under the agreement between the Governments of Australia and New Zealand on Uniform Food Standards, ANZFA is required to ensure that food standards are consistent with the obligations of both countries as members of the WTO.

In certain circumstances Australia and New Zealand have an obligation to notify the WTO of changes to food standards to enable other member countries of the WTO to make comment. Notification is required in the case of any new or changed standards which may have a significant trade effect and which depart from the relevant international standard (or where no international standard exists).

This matter will be notified to the WTO because the proposed variation to the Code constitutes a minor change that is expected to liberalise trade and therefore to impact on trade issues.

ATTACHMENT 3

DRAFT SAFETY ASSESSMENT REPORT

APPLICATION A416

Food derived from Glyphosate-tolerant Corn Line NK603

SUMMARY AND CONCLUSIONS

Glyphosate-tolerant corn line NK603 has been developed primarily for agricultural purposes to provide growers with an additional variety of corn that has been engineered for tolerance to the broad spectrum herbicide, glyphosate. A separate glyphosate-tolerant corn, line GA21, has previously undergone a safety assessment and was approved for food use in Australia and New Zealand on 24 November 2000.

1. Nature of the genetic modifications

In this application, the glyphosate-tolerance trait has been introduced into corn plants by the addition of a bacterial gene encoding the EPSPS protein, a key enzyme in the biosynthesis of aromatic amino acids in plants and microbes. The mode of action of glyphosate is to bind to the plant EPSPS protein, thereby impairing its normal enzyme activity, subsequently resulting in plant cell death. The bacterial form of the enzyme (denoted as CP4 EPSPS) has a lower affinity for glyphosate, so that when present in plant cells, the activity of the introduced enzyme replaces the sensitive plant EPSPS enzyme. The result is that the engineered plant is able to function in the presence of the herbicide.

Line NK603 contains two linked copies of the CP4 EPSPS gene, each with separate regulatory sequences. One copy is expressed from the rice actin promoter and intron while the second is expressed from the enhanced cauliflower mosaic virus promoter, which have both been shown to direct constitutive protein expression in corn. Additional regulatory sequences in common include an optimised chloroplast transit peptide sequence, to direct translocation of the CP4 EPSPS protein to chloroplasts where the protein is functionally active, and a NOS 3' untranslated region providing the appropriate eukaryotic polyadenylation signal. Because a purified segment of DNA was used in the transformation, no extraneous bacterial genes, including laboratory marker genes, were transferred.

General safety 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. The bacterial gene used in corn line NK603 is derived from a common soil bacterium, *Agrobacterium* sp. strain CP4 which is not pathogenic. Comprehensive analytical data on the modified corn is available. There is only one new protein, namely the CP4 EPSPS enzyme, produced by the genetic modification. This new protein is present in corn grain, however the family of EPSPS proteins are ubiquitous in plant and microbial food sources that are already part of human diets.

2. Toxicological issues

The chemical similarity, and functional identity, of the CP4 EPSPS protein to other EPSPS proteins already consumed as part of the human diet provide some evidence that there is no inherent toxicity associated with the introduced protein. This was supported by the results of an acute toxicity study in mice, where animals were given purified CP4 EPSPS protein at single dose levels up to 400 mg/kg. There were no clinical signs of toxicity and animals continued to grow normally for the duration of the 9 day study.

Similarly, there is no evidence to indicate that food derived from corn line NK603 would be more likely to cause allergies than food derived from the non-transformed counterpart. The CP4 EPSPS 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. In addition, there is no possibility for the transfer of marker genes to cells in the human digestive tract from the consumption of food products derived from NK603 corn as the transformation was achieved using a purified DNA segment that did not include extraneous genetic material or antibiotic resistance marker genes.

3. Nutritional issues

All parts of the grain may be used to produce food fractions including corn oil, flour, starch and sugars, particularly high fructose corn syrup. The results of extensive compositional analyses on glyphosate-treated plants grown at multiple locations demonstrate that the levels of the important components in NK603 corn grain (protein, total fat, carbohydrate, ash, fibre, fatty acids, amino acids, minerals and moisture) are not different from the non-transformed parental line. In addition, analyses for Vitamin E, phytic acid and trypsin inhibitor confirmed that the modification has not resulted in any variation to these minor components.

Statistical analysis of the results for fatty acids and amino acids showed that some minor differences between the transformed line and non-transformed control line occurred at one or two of the trial sites. However, the nature of the differences was not consistent across all sites in the two major studies and therefore the differences were considered to reflect random variation that is characteristic of large-scale plant analyses. Moreover, all compositional results from the transformed line were well within the ranges observed for commercial non-transformed lines for each of the parameters investigated.

Corn line NK603 was also shown to be equivalent to its non-transformed counterpart in the ability to support typical growth and well being when included in the diet of rapidly growing broiler chickens.

4. Conclusion

EPSPS enzymes from various plant and microbial food sources have been part of the protein component of the human diet over thousands of years, and are not associated with any known health concerns. The assessment of the safety of food derived from glyphosate-tolerant corn line NK603 is based on:

(i) a thorough understanding of the genetic modification and identification of the new gene product;
(ii) characteristics of the CP4 EPSPS protein in relation to its potential toxicity or allergenicity;
(iii) compositional analysis of the modified corn line compared to traditional corn lines.

The conclusion from this assessment is that, on the basis of the available evidence, glyphosate-tolerant corn line NK603 is compositionally equivalent to unmodified corn varieties, and is therefore suitable for human food use with respect to its safety, nutritional properties and wholesomeness.

1. BACKGROUND

Monsanto Australia Limited has submitted an application to ANZFA to vary Standard A18 of Volume 1 (Standard 1.5.2 of Volume 2) of the *Food Standards Code* to include food products derived from a glyphosate-tolerant corn line, known commercially as Roundup Ready® (RR) corn line NK603.

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). Tolerance to glyphosate in plants can therefore be achieved by introducing a bacterial 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.

The modified corn described in this application is glyphosate-tolerant corn line NK603. In this line, the glyphosate-tolerance trait is generated in the plants through the addition of a bacterial EPSPS gene derived from a common soil bacterium, *Agrobacterium* sp. strain CP4 (CP4 EPSPS). The enzyme produced from the introduced gene has a reduced affinity for the herbicide compared with the corn enzyme, and thus imparts glyphosate tolerance to the whole plant.

The bacterial CP4 EPSPS is used also in Roundup Ready® varieties of soybean, canola, sugar beet and cotton. With the exception of sugar beet, which has been assessed by ANZFA but not yet approved, foods derived from these modified crop lines have already undergone a safety assessment and have been approved[4] for food use in Australia and New Zealand under Standard A18 – Food Produced Using Gene Technology in Volume 1(Standard 1.5.2 in Volume 2) of the *Food Standards Code*.

Corn is used predominantly as an ingredient in the manufacture of breakfast cereals, baking products, extruded confectionery and corn chips. Maize starch is used extensively by the food industry for the manufacture of many processed foods including dessert mixes and canned foods.

Despite the diverse uses of corn products in many foods, corn is a relatively minor crop in both Australia and New Zealand, with a declining area planted over the last decade. Consequently, there is a requirement to import products such as high-fructose corn syrup and maize starch to meet manufacturing demand. The glyphosate-tolerance trait has not been introduced into sweet corn or popcorn varieties and therefore the whole kernel from corn line NK603 is not consumed directly as food, but rather is processed into various corn fractions.

[4] The *Food Standards Code*, refer to the Table to clause 2 of Standard A18-Food Produced Using Gene Technology (Volume1), or Standard 1.5.2 (Volume 2).

2. DESCRIPTION OF THE GENETIC MODIFICATION

2.1 Methods used in the genetic modifications

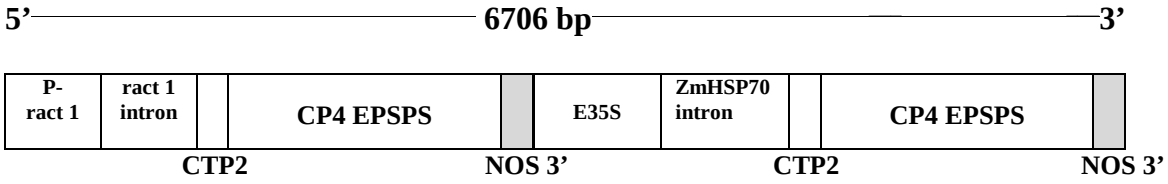
Corn line NK603 was generated by transformation of embryogenic corn (*Zea mays*) cells using a particle acceleration method. This method of transformation allowed for a specific segment of plasmid DNA, purified by gel electrophoresis and incorporating only the genes of interest together with essential controlling elements, to be transferred to the plant genome. Since the introduced DNA contained a gene encoding for herbicide tolerance (in this case, the CP4 EPSPS gene), the plant cells were grown in the presence of glyphosate and only those cells which carry the DNA modification continue to grow. The independent plant line, NK603, was subsequently developed from cultivation of the transformed corn cells.

2.2 Function and regulation of the introduced genes

A specific DNA segment of 6706 base pairs (bp) was purified from plasmid PV-ZMGT32 by agarose gel electrophoresis and subsequently used in the transformation of embryogenic corn cells. The purified fragment consisted of two adjacent gene expression cassettes, each comprising a single copy of the CP4 EPSPS gene fused to an optimised chloroplast transit peptide sequence and separate controlling DNA elements essential for expression in plant cells (see below). The segment does not contain an antibiotic resistance selectable marker gene or bacterial origin of replication sequences.

In the first (5' end) expression cassette, the CP4 EPSPS gene is under the regulation of the rice actin promoter and rice actin intron. The second cassette, which is fused to the 3' end of the first, consists of the CP4 EPSPS gene regulated by the enhanced cauliflower mosaic virus 35S promoter (e35S) and intron from the corn heat shock protein 70 (HSP70). Both expression cassettes incorporate the 3'untranslated region of the nopaline synthase gene (NOS 3') for signal polyadenylation.

Diagrammatically, the introduced DNA segment can be represented as follows:



Although plasmid PV-ZMGT32 contained other bacterial genes and controlling sequences for selection and replication in the laboratory, these sequences were not contained within the gel purified segment used in the transformation and therefore are not present in the plant.

2.2.1 CP4 EPSPS gene cassettes

Each gene expression cassette consists of the CP4 EPSPS gene fused to promoter elements required for expression in plants, and a transcription-termination element for stability of expression. The DNA components present in the expression cassettes are described below:

Genetic element	Source	Size (kb)	Function
P-ract 1/ ract 1 intron	Rice (*Oryza sativa*)	1.4	5' region of the rice actin 1 gene containing the promoter, transcription start site and first intron (McElroy *et al*., 1990).
e35S	Cauliflower Mosaic Virus (CaMV)	0.6	The 35S promoter from the cauliflower mosaic virus (Odell *et al*., 1985) with the duplicated enhancer region (Kay *et al*., 1985).
CTP2	*Arabidopsis thaliana*	0.2	DNA sequence for the chloroplast transit peptide, isolated from the *Arabidopsis thaliana* EPSPS. This component is present to direct the CP4 EPSPS protein to the plant chloroplasts where aromatic amino acid biosynthesis occurs (Klee and Rogers, 1987).
Zmhsp 70	*Zea mays* L.	0.8	Intron from the corn *hsp70* gene (heat shock protein) present to stabilise the level of transcription in plants.
CP4 EPSPS	*Agrobacterium* sp. strain CP4	1.4	The DNA sequence for CP4 EPSPS, isolated from *Agrobacterium* sp. strain CP4 which confers glyphosate tolerance (Harrison *et al*., 1993; Padgette *et al*., 1996)
NOS 3'	*Agrobacterium tumefaciens*	0.3	A 3' untranslated region of the nopaline synthase gene from the soil bacterium *Agrobacterium tumefaciens* T-DNA which ends transcription and directs polyadenylation of the mRNA (Fraley *et al*., 1983)

2.3 CP4 EPSPS gene

The bacterial CP4 EPSPS gene sequence has been shown to provide high levels of tolerance to glyphosate when it is expressed in plants (Padgette *et al*., 1993; OECD, 1999). The same gene sequence has been used to confer glyphosate-tolerance in a range of food crops namely canola, cotton, soybeans, and sugarbeet, as well as corn.

The EPSPS enzyme is a key enzyme involved in the biosynthesis of aromatic amino acids by the shikimate pathway, common to all plants, bacteria and fungi. The bacterial CP4 EPSPS protein is therefore one of many versions of the EPSPS enzyme found in nature (Schulz *et al*.,1985). However, the CP4 EPSPS protein has a high catalytic efficiency compared to most other EPSPS enzymes (Barry *et al*., 1992; Padgette *et al*. 1993 & 1996) and, in addition, is highly tolerant to glyphosate due to a lower binding affinity with that herbicide.

The mechanism of action of glyphosate is to bind specifically to the EPSPS protein, blocking the enzyme activity, and thereby interfering with normal protein synthesis in plant cells, leading to plant death. Plants that express the CP4 EPSPS gene are tolerant to glyphosate due to the continued activity of the enzyme in the presence of the herbicide, allowing normal cellular functions to continue. The CP4 and native corn EPSPS enzymes are therefore functionally equivalent, except for their affinity for glyphosate.

The two introduced CP4 EPSPS genes are respectively under the control of the enhanced cauliflower mosaic virus 35S promoter (e35S) and the actin 1 promoter from rice and therefore both copies of the gene would be expected to be expressed in all parts of the plant, conferring resistance to the herbicide at the whole plant level.

2.3.1 Chloroplast transit peptide

In both plant gene expression cassettes, the CP4 EPSPS coding sequence is fused to a chloroplast transit peptide (CTP2) whose sequence is based on the CTP isolated from *Arabidopsis thaliana* EPSPS. The purpose of the CTP is to direct the new protein to the chloroplast, where the enzymes of the shikimate pathway operate in plant cells, and therefore where the endogenous corn EPSPS enzyme is naturally transported.

Transit peptides are commonly occurring molecular mechanisms to facilitate intracellular transport of proteins between compartments within a cell. The CTP is typically cleaved from the mature protein on uptake into the chloroplast, and subsequently rapidly degraded.

2.4 Characterisation of the genes in the plant

Studies submitted:

Deng, M.Y., Lirette, R.P., Cavato, T.A. and Sidhu, R.S.. Molecular characterisation of Roundup Ready® (CP4 EPSPS) Corn Line NK603. Monsanto Laboratory Project 99-01-46-26, MSL – 16214, completed October 1999.

Cavato, T.A., Deng, M.Y. and Lirette, R.P.. Confirmation of the Genomic DNA Sequences Flanking the 5' and 3' Ends of the Insert in Roundup Ready® Corn Event NK603. Monsanto Laboratory Project 00-01-46-30, MSL – 16857, completed October 2000.

Silanovich, A., Hileman, R.E., and Astwood, J.D.. Amended Report for Bioinformatic Evaluation of DNA Sequences Flanking the 3' End of the NK603 Insertion Event: Assessment of Putative Polypeptides. Monsanto Laboratory Project 00-01-46-41, MSL – 17005, completed October 2000 (Amendment 1 completed December 2000).

Multiple molecular analyses were undertaken in order to characterise the inserted DNA in corn line NK603. Genomic plant DNA was analysed using the standard methodology of Southern blot analysis to determine the insert number and the copy number as well as to provide information about the integrity of the inserted regulatory sequences and to confirm the absence of any of the plasmid backbone sequences. Polymerase chain reaction (PCR) methodology was used to verify the sequences at the ends of the inserted DNA segment at the points where it is integrated into the plant DNA.

The test material used was leaf tissue taken from corn line NK603 grown under greenhouse conditions and treated with Roundup Ultra® (64 ounces/acre) at the V2-V3 stage (2-3 leaf collars). Leaf tissue from the untransformed parental corn line LH82 x B73 grown under similar conditions was used as the control material.

Data from the analyses support the following conclusions. Firstly, the genome of corn line NK603 contains a single DNA insertion as determined by multiple Southern blot analyses using different known molecular cleavage sites within the region of the introduced segment. Secondly, Southern blot analysis using different DNA probes supports the conclusion that there is one complete copy of the DNA segment used in the transformation. This result is consistent with and further supports the first conclusion that a single insertion exists in corn line NK603.

Thirdly, as well as the single complete copy of the DNA segment used in the transformation, the insertion also includes a non-functional, inversely linked 217 bp fragment of the enhancer region of the rice actin promoter at the 3'end of the introduced DNA. The evidence for this was provided by Southern blot analysis and DNA sequence analysis of the regions at the ends of the inserted segment.

The sequence data revealed that the 217 bp fragment includes polylinker sequence (50 bp) and the first 167 bp of the enhancer region of the rice actin promoter. However, from previously published studies on the rice actin promoter and intron, it is known that essential elements necessary for promoter function are not present in these 167 additional nucleotides (McElroy *et al.*, 1990).

2.4.1 Verification of the 5' and 3' flanking sequences

Polymerase chain reaction (PCR) methodology was used on genomic DNA extracted from leaf tissue from corn line NK603 to verify the nucleotide sequence at the 5' and 3' ends of the newly inserted segment. This approach provides unequivocal data on the nature of the inserted DNA segment present in the transformed line and supplements the information obtained by the multiple Southern hybridisation analyses carried out using different molecular probes. DNA extracted from the leaves of a non-transformed line B73 was used as a control in the PCR experiments.

Using primers specific for the known regions at the ends of the inserted DNA, PCR products were generated, subsequently cloned and the nucleotide sequence determined. The sequence data confirm that the transformation cassette is intact at the 5' end of the inserted DNA. These data also show that, at the 3' end, a portion of the enhancer region of the rice actin promoter is linked in the opposite orientation to the transformation cassette. These results confirm, as predicted by the molecular characterisation, that one complete copy of the DNA segment used for transformation is present in the corn genome, together with an additional 217 bp corresponding to a non-functional portion of the rice actin promoter.

Additional DNA sequence

The nucleotide sequence data also determined that a segment of chloroplast DNA (305bp) is immediately adjacent to the 3' end of the introduced DNA segment. It is apparent that this additional DNA has co-integrated with the transformation cassette at the same time. Bioinformatic analysis unequivocally identified the fragment of chloroplast DNA as corresponding to the coding sequence for the α-subunit of chloroplast DNA-directed RNA polymerase and ribosomal protein S11 in maize. This extraneous DNA was not present in the gel-isolated segment used in the transformation process and therefore the origin of the chloroplast DNA was the transformed, embryonic maize cell itself.

2.4.2 Summary and conclusions from sequence analysis

The sequence data define the 5' and 3' ends of the insertion event in corn line NK603 and provide corn genomic sequence extending to approximately 300 nucleotides upstream and 500 nucleotides downstream of the introduced segment. At the 3' end of the introduced DNA, as well as the minor rearrangement of the transformation cassette, additional extraneous DNA is present, derived from the corn chloroplast DNA.

Given the method of transformation used to generate this line, some DNA rearrangements would reasonably be expected as these have been commonly observed in plant transformations and are often reported in the scientific literature. The rearrangements do not necessarily raise any public health or safety concerns provided that they are fully characterised using detailed molecular and bioinformatic tools. In this case, the analyses are comprehensive and indicate that the additional DNA sequence at the 3' end is non-functional on the basis of previously published observed sequence requirements of the enhancer region of the rice actin promoter (McElroy *et al*., 1990 & 1991).

Furthermore, there are several published examples where host genetic material has been observed to co-integrate with transgenes at the site of integration. It is most likely that this results from the normal DNA repair mechanisms naturally found in living cells and which are utilised deliberately and effectively in the plant transformation process. In corn line NK603, the multiple genetic and bioinformatic analyses that have been applied to the integrated chloroplast DNA and surrounding regions, show no particular characteristics that would be likely to raise food safety concerns. The detailed bioinformatic investigation of putative peptides arising from the presence of the chloroplast DNA revealed no relevant sequence similarity to known toxins or allergens. The DNA corresponds to the native genetic material of the corn plant and, despite its non-native location in this plant line, has always been a natural part of this food.

2.5 Stability of the genetic changes

The stability of the transferred genes was investigated to ascertain plant characteristics over multiple generations. Statistically analysed segregation data for nine generations was presented by the applicant, based on the frequency of observed versus expected numbers of progeny with tolerance to glyphosate. The stability of the insert was demonstrated through six generations of crossing and three generations of self pollination. These data show that the herbicide tolerance trait in corn line NK603 is inherited according to predicted patterns, consistent with a single active site of insertion of the CP4 EPSPS into the genomic DNA, segregating according to Mendelian genetics.

Southern blot analysis was also conducted to assess the genetic stability of the inserted DNA in this line including, as controls, non-transformed B73 corn DNA and the same B73 DNA spiked with the original plasmid DNA. Genomic DNA extracted from leaf tissues of the F1 generation (the progeny from a R0 back cross) and the fifth generation of back-crossing (BC5F1) of line NK603 and both control samples were appropriately cleaved, and probed with the full-length CTP2-CP4 EPSPS fragment.

There were no detectable differences in the observed banding pattern between the DNA extracted from the F1 generation and from the BC5F1 generation. These results demonstrate that the integrated segment in corn line NK603 is stable spanning five generations.

2.6 Conclusion

Corn line NK603 was produced using the particle acceleration method with a linear DNA segment comprised of two linked CP4-EPSPS gene cassettes, each regulated by a different promoter. A plant promoter from rice is used in the first gene cassette while a promoter from the commonly occurring cauliflower mosaic virus is used in the second expression cassette. Other regulatory elements are common to both gene cassettes and have been used in certain other genetically modified plants, including some that have previously undergone a safety assessment and have been subsequently approved for listing in the standard for foods produced using gene technology. The molecular characterisation of this line involved multiple analyses using Southern blot hybridisations, PCR and nucleotide sequencing.

At the molecular level, the analyses indicate that the transformation process has resulted in a single insertion event, comprising one complete copy of the transformation cassette together with an additional small fragment of the enhancer region of the rice actin promoter linked at the 3' end of the inserted DNA in an inverse orientation. In addition, approximately 300 bp of chloroplast DNA has co-integrated during transformation. The additional nucleotides are completely identified and are expected to be non-functional on the basis of previously published studies delineating the minimum sequence requirements for functionality. The data further indicate that the inserted DNA is physically stable and is inherited in a predictable manner over multiple generations.

3. GENERAL SAFETY ISSUES

3.1 History of use

Recipient organism

The crop species modified in this application is corn, *Zea mays* L., also known as maize. Corn 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. 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 a major crop, it is the principal component of livestock feeds, and most of it is fed to farm animals, particularly to ruminants. The use of corn as a major constituent of human diets is limited to only a few countries. 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, 1999). 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.

Donor organism

The only new gene expressed in the corn plants, the CP4 EPSPS, is derived from the bacterial species *Agrobacterium* sp. strain CP4. The bacterial isolate, CP4, was identified by the American Type Culture Collection as an *Agrobacterium* species, commonly found in soil. This species is not known to be pathogenic to either humans or animals. The native corn EPSPS and the CP4 EPSPS are functionally equivalent except for the binding affinity for glyphosate which is significantly reduced in the bacterial form of the enzyme.

3.2 Nature of novel protein

Studies submitted:
Padgette, S.R., Barry, G.F., Re, D.B., Weldon, M., Eichholtz, D.A., Kolacz, K.H. and Kishore, G.M., 1993. Purification, Cloning and Characterisation of a Highly Glyphosate Tolerant EPSP Synthase from *Agrobacterium* sp. strain CP4. Monsanto Technical Report MSL-12738, St. Louis, Missouri.

As part of the safety assessment of glyphosate-tolerant corn line NK603, the assessment examines the expressed products of the introduced genes and considers the levels of new protein in the grain. In this line, the only expressed protein product from the inserted gene cassettes is the CTP2-CP4 EPSPS protein, the other DNA elements being controlling sequences.

The EPSPS enzyme catalyses a non-rate limiting step in the shikimate pathway involved in aromatic amino acid biosynthesis in plants and microorganisms (Steinruken and Amrheim, 1980). Since EPSPS 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 (see Schultz *et al*., 1985 and Barry *et al.*, 1992). Data from these studies show that differences in amino acid sequence of the enzyme from different species, including bacteria and fungi, result in varying degrees of sensitivity to glyphosate. The bacterial CP4 version of the EPSPS enzyme introduced into corn line NK603 exhibits a lower binding affinity for glyphosate and thus exhibits high catalytic efficiency in the presence of glyphosate when compared to the native corn EPSPS.

The catalytic function of the introduced CP4 EPSPS enzyme is well characterised in plants. It has been established that CP4 EPSPS is highly specific for its natural substrates, shikimate-3-phosphate and phosphoenolpyruvate, similar to the corn enzyme (Padgette *et al*., 1993; Gruys and Sikorski, 1999). The characterisation included an examination of three dimensional folding patterns of the protein and sequence homology at the active site enabling comparison with the structure and function of the native corn EPSPS. The shikimate pathway does not occur in mammals, where aromatic amino acids are provided from other sources, a fact contributing to the selective toxicity of glyphosate to plants.

The CP4 EPSPS has been completely sequenced and encodes a 47.6 kDa protein consisting of a single polypeptide of 455 amino acids (Padgette *et al*., 1996). The deduced amino acid sequence of the CP4 EPSPS with the CTP2 transit peptide (amino acids 1-76) was provided as part of the data package submitted in support of this application.

The bacterial enzyme exhibits approximately 50% amino acid sequence similarity with plant EPSPS enzymes (eg. soybean, corn and petunia).

The degree of similarity of the CP4 EPSPS protein to other EPSPS enzymes naturally present in all food crops (eg. soybean and corn) and in fungal and microbial food sources such as Baker's yeast (*Saccharomyces cerevisiae*) and *Bacillus subtilis* (Mountain, 1989) which have been safely consumed by humans for centuries, is evidence that this family of proteins has been an integral part of the food supply throughout history.

3.3 Protein expression

Studies submitted:

Bisop, B.F., 1993. Production of CP4 EPSP synthase in a 100 litre recombinant *Escherichia coli* fermentation. Monsanto Technical Report MSL-12389, St Louis, Missouri.

Harrison, L.A., Leimgruber, M.R., Smith, C.E., Nida, D.L., Taylor, M.L., Gustafson, M., Heeren, B. and Padgette, S.R., 1993. Characterisation of Microbially-Expressed Protein: CP4 EPSPS. Monsanto Technical Report MSL-12901, St Louis, Missouri.

Ledesma, B.E. and Sidhu, R.S., 1999. Development and validation of a direct ELISA for quantitation of CP4 5-enolpyruvylshikimate-3-phosphate synthase (CP4 EPSPS) protein in corn tissues from Roundup Ready® plants. Monsanto Technical Report, MSL-16259, St. Louis, Missouri.

Lee, T.C. and Astwood, J.D., 1999. Assessment of the Equivalence of CP4 EPSPS Protein Expressed in *Escherichia coli* and in Roundup Ready® corn lines NK600 and NK603. Monsanto Technical Report MSL-16392, St Louis, Missouri.

Under the regulation of the rice actin promoter (expression cassette 1) and the 35S promoter (expression cassette 2), the new protein is expected to occur throughout the whole plant, including the grain, since these promoters have been shown to drive constitutive gene expression in genetically modified corn. The applicant has submitted several studies conducted to characterise the expressed protein and to determine the level of novel gene expression in corn line NK603 by various methods including Western blot analysis (immunoblotting) and an enzyme-linked immunosorbent assay (ELISA).

3.3.1 Western blot analysis

The expression of the full-length CP4 EPSPS protein in the grain from corn line NK603 was confirmed by Western blot analysis. Two control materials were used for this study. The first control material was obtained from a non-transformed parental corn line (LH82xB73) that does not contain the genetic material to encode CP4 EPSPS. Grain for the transformed and non-transformed corn lines was collected from field grown plants. The second control material was a non-transformed soybean line, A5403, which likewise does not express CP4 EPSPS. The presence or absence of the CP4 EPSPS gene in the tested lines was established by a Polymerase Chain Reaction (PCR) detection method.

Two reference materials were also used for this study. The primary reference material was *in vitro* produced CP4 EPSPS derived from recombinant *E. coli*, transformed with a plasmid encoding the enzyme. The applicant used a second reference material which was CP4 EPSPS endogenously expressed by a similarly transformed soybean line, AG3701, obtained from Asgrow (Stonington, Illinois).

This soybean line is glyphosate-tolerant due to the presence and expression of the bacterial CP4 EPSPS gene, also present in corn line NK603. Immunoblotting involved the use of polyclonal antisera raised in goats against the *E. coli* produced CP4 EPSPS protein.

The results of the Western blot analysis demonstrated that the *E. coli* produced CP4 EPSPS protein used for the safety studies, the CP4 EPSPS expressed in the glyphosate-tolerant soybeans and the CP4 EPSPS expressed by glyphosate-tolerant corn line NK603 were identical, based on electrophoretic mobility and detection using specific antibodies. Immunoreactive bands at the expected apparent molecular weight (approx. 47 kDa) were observed for all CP4 EPSPS-containing samples, whether *E. coli* produced protein or extracted from the transformed corn or soybean plants. No immunoreactive bands were detected in the control (untransformed) corn or soybean extracts, confirming the specificity of the antibodies in detecting the expressed protein.

3.3.2 ELISA detection

Levels of the CP4 EPSPS protein were estimated in both forage and grain samples collected from six non-replicated and two replicated field sites, representative of the major U.S. corn production region during the 1998 growing season. Samples collected from line NK603 and the non-transformed parental control line (LH82xB73) were analysed using ELISA. The CP4 EPSPS protein levels in forage and grain extracts were estimated using a double antibody sandwich ELISA consisting of a monoclonal anti-CP4 EPSPS antibody as the capture antibody and a polyclonal anti-CP4 EPSPS conjugated to horseradish peroxidase (HRP) as the detection antibody. The CP4 EPSPS protein levels in plant tissue extracts were quantified by comparison of the sample absorbance (OD) to the absorbance produced by a range of concentrations of purified CP4 EPSPS reference standard. This protein standard was purified in the laboratory from an *E. coli* strain expressing the *Agrobacterium* sp. strain CP4 EPSPS, and fully characterised in the applicant's study (Harrison *et al*., 1993).

The CP4 EPSPS protein levels estimated in corn forage and grain samples are summarised in Table 1. The levels of CP4 EPSPS protein in all non-transformed control samples were below the limit of quantitation (LOQ) of the assay (data not presented).

Table 1. Summary of CP4 EPSPS protein levels measured by ELISA in tissues of NK603 corn plants (µg/g fresh weight). The LOQ for forage equals 0.05 µg/g fw, and for grain equals 0.09 µg/g fw.

Sites	Parameter	Forage µg/g fw	Grain µg/g fw
Non-replicated	Mean	25.5	11.0
	Range	18.0-31.2	6.9-15.6
	SD	4.5	3.2
Replicated	Mean	25.9	10.6
	Range	25.7-26.1	9.8-11.3
	SD	0.3	1.0
All sites	Mean	25.6	10.9
	Range	18.0-31.2	6.9-15.6
	SD	3.8	2.6

SD = Standard Deviation

As expected, the results of the ELISA show that mature CP4 EPSPS is present in low concentrations in the grain and at higher concentrations in the forage of corn line NK603. A higher level of novel protein expression in the green tissues of the plant (corresponding to the forage) is consistent with the functional rice actin and viral promoters used in the gene constructs. Although the level of expression is low, it is sufficient to confer tolerance to glyphosate at the level of the whole plant. The mean CP4 EPSPS protein levels in NK603 grain were comparable at the non-replicated sites (11.0 µg/g fw) and the replicated sites (10.6 µg/g fw) indicating that the novel protein is expressed at approximately the same levels either within a site or across geographically dispersed sites.

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

The human health considerations in relation to the potential for horizontal gene transfer 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[5]/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 potential 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 in the laboratory or in the field. 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 this application, the transformation method allowed for a gel-purified specific segment of plasmid DNA to be used to transform the plant cells from which corn line NK603 was subsequently generated. The DNA segment corresponded only to the gene of interest in conjunction with the essential controlling elements. Consequently, no extraneous plasmid DNA sequences such as antibiotic resistance marker genes were ever introduced into this plant line. In this case, positively transformed plant cells were selected using the introduced glyphosate-tolerance trait.

3.5 Conclusion

The one novel protein expressed in corn line NK603 is CP4 EPSPS, a bacterial form of an enzyme already naturally occurring in all plants including corn. Analysis of the modified line indicates that the new protein is present both in the grain, the part of the plant used as food, and the forage which is not used for human consumption. No laboratory marker genes, in particular antibiotic resistance genes, were transferred during the plant transformation process.

[5] Food and Agriculture Organization.

4. TOXICOLOGICAL ISSUES

The family of EPSPS proteins are naturally present in foods derived from plants and microbes and have no known history of toxicity or allergenicity.

The safety of other foods derived from modified crops containing the CP4 EPSPS protein used in this application has been previously addressed in assessments of glyphosate-tolerant soybeans, insect-protected corn, glyphosate-tolerant cotton and glyphosate-tolerant canola. Studies that are of some relevance to an assessment of the potential toxicity and allergenicity of this protein in the context of other GM foods have been published in the scientific literature (for example, Harrison *et al*., 1996; Hammond *et al*., 1996).

4.1 Levels of naturally occurring toxins

More than 70% 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 fungal contamination of corn kernels as a result of production or storage under adverse conditions. They are not a natural component of sound corn.

4.2 Potential toxicity of newly expressed protein

The detailed protein expression analyses have demonstrated that the only new protein present in corn line NK603 is the bacterial CP4 EPSPS enzyme. The CP4 EPSPS gene has been completely sequenced and encodes a 47.6 kDa protein consisting of a single polypeptide of 455 amino acids. At the amino acid level, this enzyme is similar to other EPSPS enzymes in this family of proteins with a function common to plants and microorganisms. The similarity of the CP4 EPSPS protein to other EPSPS proteins naturally present in a variety of human foods derived from plants (for example, soybean and tomato) and microbes (for example, Baker's yeast and *Bacillus subtilis*) provides supporting evidence for the safety of this protein.

4.2.1 Sequence comparison to known toxins

Study: Hileman, R.E. and Astwood, J.D. (1999b). Bioinformatics Analysis of CP4 EPSPS Protein Sequence Utilising Toxin and Public Domain Genetic Databases. Monsanto Technical Report MSL-16268, St. Louis, Missouri.

EPSPS proteins from plants and other biological sources have a long history of consumption by humans and have not been associated with toxicity in relation to human health. However, to specifically test the bacterial CP4 EPSPS for potential toxicity, a range of analyses was completed by the applicant.

A database of 4,677 protein sequences (not all unique) associated with toxicity was assembled from publicly available genetic databases such as PIR, SwissProt, EMBL and GenBank. The amino acid sequence of the CP4 EPSPS protein was compared to protein sequences in the toxin database using the FASTA[6] sequence alignment tool.

[6] FASTA is based on the algorithms of Needleman and Wunsch (1970) and of Smith and Waterman (1981), which consider all possible alignments between a query sequence and a database sequence.

In addition, the amino acid sequence of the CP4 EPSPS protein was compared to all protein sequences in the publicly available sequence databases to screen for structural similarity to other known proteins, including pharmacologically active proteins. As expected from prior examinations and comparisons, the CP4 EPSPS protein shares sequence similarity only with other EPSPS proteins from different biological sources. These computer searches did not reveal other significant structural homology, confirming the lack of similarity of the CP4 EPSPS protein to known protein toxins.

4.2.2 Acute oral toxicity study in mice

Studies submitted:
Harrison, L.A., Bailey, M.R., Leimgruber, R.M., Smith, C.E., Nida, D.L., Taylor, M.L., Gustafson, M.E., Heeren, B. and Padgette, S.R. (1993) Characterisation of Microbially-Expressed Protein: CP4 EPSPS. Monsanto Technical Report MSL-12901, St Louis, Missouri.

Heeren, R.A., Padgette, S.R. and Gustafson, M.E. (1993). The purification of recombinant *Escherichia coli* CP4 5-enolpyruvyl-shikimate-3-phosphate synthase for equivalence studies. Monsanto Technical Report MSL-12574, St Louis, Missouri.

Naylor, M.W. (1993). Acute Oral Toxicity Study of CP4 EPSPS Protein in Albino Mice. Monsanto Technical Report MSL-13077, St Louis, Missouri.

As a further test for potential toxicity, the applicant carried out an acute oral toxicity study of CP4 EPSPS in young laboratory mice using purified (>90%) protein produced in *E. coli* in the laboratory. A separate protein characterisation study was completed in order to confirm the equivalence of the bacterially produced enzyme used in the toxicity study to the protein expressed in the modified plants. The results of the study showed that the purified CP4 EPSPS exhibits the appropriate chemical identity and integrity as determined by gel electrophoresis, Western blot (immunoblotting), N-terminal amino acid sequencing and ELISA. The purified protein also demonstrated functional identity as determined by enzymatic activity.

The study was conducted in general compliance with the EPA FIFRA (40 CFR Part 160). A total of 100 animals (50 males and 50 females) were used in this study, ranging from 5.5 weeks to 7 weeks of age. Test groups were randomised for weight and comprised 10 CD-1 mice of each sex per group. The protein preparation containing the CP4 EPSPS was administered as a single dose by gavage to three groups of the mice at dosages of 49, 154 and 572 mg/kg body weight respectively. These doses correspond to 40, 100 and 400 mg/kg of CP4 EPSPS protein based on the level of purity of the protein and ELISA analyses of the dosing solutions. A control group received bovine serum albumin (BSA) at a dosage of 363 mg/kg in the same solution and delivery volume as the test substance. The second control group was administered the carrier solution only, 50 mM sodium bicarbonate.

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. Signs of toxicity include such occurrences as changes in the skin and fur, eyes and mucous membranes, respiratory, autonomic and central nervous systems as well as behavioural changes. At the termination of the study (day 8-9), animals were sacrificed, examined for gross pathology and numerous tissues were collected.

Tissues retained from the animals included aorta, adrenals, brain, colon, oesophagus, eyes, gall bladder, heart, kidneys, lung, liver, lymph nodes, muscle, ovaries, pancreas, pituitary, prostate, rectum, salivary gland, seminal vesicles, skin, spinal cord, spleen, stomach, testes, thymus, uterus and bladder. Hollow organs were opened and examined.

The results of the study showed no statistically significant differences in group mean body weights, cumulative weight gains or food consumption in any of the groups treated with either BSA or the CP4 protein, when compared with the carrier control group. The data were evaluated according to a decision-tree analysis procedure which, depending on the results of early statistical tests, determined further statistical analysis applied to detect group differences and analyse for trends. All animals survived to the scheduled termination of the study, and there were no clinical signs observed that could be related to the test material.

As recorded in Table 2, a small number of general pathological observations were detected in the female mice but these occurred throughout all groups in the study, including both control groups that did not receive the test material, and therefore these findings cannot be related to the treatment. There were no such findings in the male animals in any of the test or control groups in the study.

Table 2. Pathology - Incidence of individual gross necropsy alterations, females.

	Carrier Control N=10	**BSA Control** N=10	**CP4 EPSPS 40 mg/kg** N=10	**CP4 EPSPS 100 mg/kg** N=10	**CP4 EPSPS 400 mg/kg** N=10
Eye (corneal opacity)	0	0	0	1	0
Kidney (cyst)	0	0	1	0	0
Pituitary (focus)	1	0	0	0	0
Uterus (hydrometra)	2	1	1	1	2

In conclusion, there was no evidence of acute toxicity in mice following a single oral dose of up to 400 mg/kg of CP4 EPSPS protein. This dose level is far in excess of the level of exposure expected from the consumption of modified corn.

4.3 Potential allergenicity of new protein

Although many foods have been reported to cause allergies in some people, the prevalence of food allergy using prospective, population-based studies has been shown to be less than 2% of adults and 2-7% of infants and children, excluding cases of food intolerances such as enzyme deficiencies. Food allergies are primarily due to an immune reaction to a particular protein or glycoprotein component of the food (FAO, 1995).

The assessment of potential allergenicity is based on a weight-of-evidence approach where certain biochemical and physical properties of the novel protein are investigated. A risk profile for the protein can then be considered on the basis of the accumulated data.

The potential allergenicity of the new protein introduced into corn line NK603 has been assessed by comparing particular molecular and biochemical properties of the new protein to those of known allergens. These include amino acid sequence similarity with known protein allergens, poor digestibility and resistance to processing. Other factors that are taken into account and that may increase the likelihood of allergic oral sensitisation to proteins include the level of food consumption, and the relative quantity of the protein in the food.

4.3.1 Digestibility of CP4 EPSPS

Study :
Ream, J.E., Bailey, M.R., Leach, J.N. and Padgette, S.R., 1993. Assessment of the *in vitro* digestive fate of CP4 EPSP synthase. Monsanto Technical Report MSL-12949.

Typically, most food allergens tend to be stable to the peptic and acidic conditions of the digestive system if they are to reach and pass through the intestinal mucosa to elicit an allergic response (Kimber *et al*., 1999; Astwood *et al*., 1996b; Metcalfe *et al*., 1996). To address the question of potential allergenicity, the applicant has investigated the physicochemical properties of the CP4 EPSPS protein, which is expressed in the corn grain at low levels, and tested its susceptibility to proteolytic degradation.

Simulated mammalian gastric and intestinal digestive mixtures (described in the United States Pharmacopoeia, 1990[7]) were established to assess the susceptibility of the CP4 EPSPS protein to *in vitro* proteolytic digestion. The protein was incubated at approximately 37°C in simulated mammalian gastric fluid (SGF) and simulated intestinal fluid (SIF). At defined periods, the digestions were terminated and the levels of remaining CP4 EPSPS protein were determined by Western blot analysis and enzymatic activity assays.

The results show that CP4 EPSPS protein degraded readily in both simulated gastric and intestinal fluids, indicating that it would similarly break down during the processes involved in human digestion. Western blot analyses demonstrated that the half–life of the protein was less than 15 seconds in the gastric system. The results of the activity assay confirmed that the activity of the enzyme had decreased by greater than 84% at the first timepoint, that is after 2 minutes incubation. There was a strong correlation between the results of the Western blot analysis and the enzymatic activity assay in the SGF experiments, providing evidence that the protein degrades rapidly in the stomach when ingested by mammals as a component of food.

In simulated intestinal fluid, the half-life of the CP4 EPSPS protein was less than 10 minutes as determined by Western blot analysis. In addition, the enzyme activity had decreased by greater than 94% after approximately 4.5 hours incubation. Overall, these digestibility results show that the introduced protein in corn line NK603 is readily degraded in a simulated digestive system and similarly readily degraded in the conditions of the mammalian digestive tract.

4.3.2 Sequence comparison to known allergens

Study submitted:
Hileman, R.E. and Astwood, J.D., 1999a. Bioinformatics Analysis of CP4 EPSPS Protein Sequence Utilising an Allergen Database, Monsanto Technical Report MSL No. 16267, St. Louis, MO.

[7] The United States Pharmacopoeia, 1990, Volume XXII, NF XVII. United States Pharmacopeial Convention, Inc., Rockville, MD, page 1788.

A comparison of the amino acid sequence of an introduced protein with the amino acid sequence of known allergens is a further useful indicator of the potential for allergenicity, based on the identification of contiguous identical sequence matches which may be immunologically significant.

A database of 567 protein sequences associated with allergy and coeliac disease was assembled from publicly available genetic databases (GenBank, EMBL, PIR and SwissProt) and from current literature. The applicant compared the amino acid sequence of the introduced CP4 EPSPS protein to these assembled sequences using the sequence alignment tool FASTA (see earlier discussion on potential toxicity). The results of the alignment showed that CP4 EPSPS shared no structurally significant sequence similarity to sequences within the assembled allergen database.

In addition, the amino acid sequence of the CP4 EPSPS protein was compared to the allergen database using an algorithm that scans for a window of eight identical linearly contiguous amino acids. This comparison did not find any sequence identities between the introduced protein and the database sequences.

4.3.3 Abundance of CP4 EPSPS

Most allergens are present as major protein components in a specific food, typically ranging between 1% and 80% of total protein (Astwood and Fuchs, 1996). In contrast, CP4 EPSPS protein is present at approximately 0.01% of the total protein found in the grain of corn line NK603, noting that the grain is composed predominantly of carbohydrate and that protein normally comprises approximately 20%-25% of the grain. Corn flour is therefore the major food product likely to include corn proteins while corn oil and corn syrup are not expected to contain plant proteins including the introduced CP4 EPSPS protein.

4.4 Summary and conclusions

The CP4 EPSPS protein is structurally and biochemically similar to other EPSPS enzymes from various plant and microbial food sources that are currently part of the human diet and have been consumed over a long period without any health concerns. The protein does not exhibit sequence similarity with known toxins and allergens, and does not exhibit the biochemical characteristics of known protein allergens. When fed as a single dose to laboratory mice at levels greatly exceeding the likely human level of exposure through consumption of whole corn grain or flour, there was no evidence of acute toxicity. Furthermore, the protein is present in relatively low abundance in the grain and demonstrates digestive lability in conditions that mimic human digestion. The data and analyses investigating the potential for toxicity and allergenicity of the novel protein therefore strongly support the conclusions that corn line NK603 expressing CP4 EPSPS does not pose any greater food safety risk than conventional corn.

5. NUTRITIONAL ISSUES

Studies submitted:

Sidhu, R.S. and Ledesma, B.E., 1999. Introduced Protein Levels and Compositional Analyses of Roundup Ready® Corn Line NK603 Tissues Produced in 1998 U.S. Field Trials. Monsanto Technical Report MSL-16278, St. Louis, MO.

Ridley, W.P., George, C., Nemeth, M.A., Astwood, J.D., Breeze, M.L.* and Sorbet R.**, 2000. Compositional Analyses of Forage and Grain Collected From Roundup Ready® Maize Event NK603 Grown in 1999 E.U. Field Trials. Monsanto Technical Report MSL-16897, St Louis, MO.
* Covance Laboratories, Madison, Wisconsin.
** Statistical Analysis Facility, Certus International Inc., Chesterfield, MO.

The key nutrients in corn have been evaluated in order to compare equivalent data from the transformed line NK603, the non-transformed counterpart and published literature ranges obtained for conventional varieties of corn. This evaluation includes a study of the major constituents that are characteristic of whole corn grain, taking account of the natural variation in composition that is known to occur due to genetic variability and multiple environmental factors.

5.1 Compositional analyses

The applicant has conducted two major studies to determine the compositional profile of key corn tissues collected from corn line NK603, the non-transformed parental control line and a series of commercial corn hybrids grown under field conditions. Trial sites were selected across the United States corn-growing belt and in multiple sites across Europe. The U.S. sites included two replicated sites in Illinois and Ohio and six non-replicated sites in Iowa, Illinois, Indiana and Kansas. The European sites included four replicated sites located in Germignonville, Janville and L'isle Jourdain in France and Bagnarola, Italy. These sites provided a breadth of environmental conditions representative of regions where corn varieties are grown as commercial products.

Sample preparation and collection

Grain and forage samples of line NK603, treated with glyphosate herbicide (application rates supplied), and the non-genetically modified parental control line together with other commercial hybrids were collected from the range of sites. In the U.S. trials, several glyphosate-tolerant corn lines, including NK603, as well as the control line were planted at each site. Five different non-transformed commercial reference hybrids were planted at each of the European sites. The test and control substances were characterised at the molecular level by extracting DNA from grain tissue and analysing the DNA by event specific polymerase chain reaction (PCR) techniques.

In general, forage was collected at the late dough/early dent stage by dividing approximately 12 randomly selected plants into three roughly equal segments and placing them on dry ice within 10 mins of collection. Ears were harvested from approximately 12 self-pollinated plants at normal kernel maturity (<32% moisture), dried to a moisture between 10-20%, shelled and the kernels pooled to provide the grain sample. Forage (stored on dry ice) and grain (stored at ambient temperature) samples were then transferred to the laboratory for compositional analyses[8] and for estimation of CP4 EPSPS protein levels (see section 3.3 - Protein expression).

To conduct the analyses, forage and grain samples were collected from glyphosate-tolerant corn line NK603, the non-transformed parental control line (LH82 x B73) and reference hybrids. The control line has the same genetic background as that of the test line but lacks the gene encoding CP4 EPSPS protein.

[8] Covance Laboratories Inc., Wisconsin Facility, 3301 Kinsman Blvd., Madison, WI 53704.

Compositional analyses included measurement of proximates (protein, fat, ash, moisture), acid detergent fibre (ADF), neutral detergent fibre (NDF), amino acids, fatty acids, vitamin E, minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium and zinc), phytic acid and trypsin inhibitor content of the grain. Forage samples were analysed for proximate, ADF and NDF content. Carbohydrate values in both forage and grain were estimated by calculation.

Although the applicant provided detailed results from the compositional analyses of both the grain and forage on a site-by-site basis, not all of the results are presented here. Moreover, as the forage is not consumed by humans, the focus of this assessment was primarily on the results obtained from analyses of the grain samples. The forage data provide supporting evidence of the lack of significant differences in composition throughout the transformed and non-transformed plants, including tissues of the plants that are not part of the human diet.

Statistical analysis

The compositional data from the U.S. field trials were developed and statistically analysed as three sets of comparisons: analyses for each of the two replicated trials and for a combination of trials at different field sites. Similar multiple statistical analyses were applied to the European trial data. The test event, NK603, was compared to the non-transformed control line to determine statistically significant differences at $p<0.05$. In addition, the comparison of NK603 to the 95% tolerance interval for the commercial reference varieties was conducted to determine if the range of values for NK603 fell within the population of commercial corn. The data presented here are summary data pertaining to all trials.

Analytical methods

The applicant provided detailed information relating to the methods used in the generation of the compositional data. For each parameter tested, as well as appropriate technical references, the limit of detection or quantitation was also stated. Methodology was predominantly derived from established references such as AACC (American Association of Cereal Chemists), AOAC (Association of Official Analytical Chemists), AOCS (American Oil Chemists Society), the USDA Agricultural Handbook (United States Department of Agriculture), or from a range of published literature methods.

5.1.1 Proximate analysis – U.S. study

The results obtained for the proximate analysis (including fibre) of the grain taken from all trial sites are presented in Table 3. The difference in the mean value for the moisture content between line NK603 and the control line was statistically significant (p-value 0.037), although values were within the commercial range for corn. This difference is not considered to be of biological significance and does not adversely affect the overall nutritional qualities or safety of food derived from the transformed corn line.

There was no statistically significant difference between the transformed corn line and the non-transformed control for the remaining proximate analyses. Furthermore, all values were within the measured range for conventionally produced commercial lines of corn and, where comparable data were available, were also within the range reported in the general literature (Watson, 1987).

Table 3: Summary of Proximate and Fibre Analysis from all U.S. trial sites.

Component	NK 603 corn Mean ± S.E.[a] (Range)	Control Mean ± S.E. [a] (Range)	Commercial[b] (Range)
Ash (% dw)	1.44 ± 0.032 (1.28 – 1.75)	1.49 ± 0.032 (1.32 – 1.75)	(0.8 – 1.8)
Carbohydrates (% dw)	82.59 ± 0.39 (80.71 – 84.33)	82.26 ± 0.39 (80.23 – 83.70)	(83.1 – 89.6)
ADF (% dw)	3.79 ± 0.16 (3.14 – 5.17)	3.70 ± 0.16 (2.79 – 4.28)	(2.3 – 5.7)
NDF (% dw)	10.38 ± 0.67 (7.89 – 12.53)	10.32 ± 0.67 (8.25 – 15.42)	(8.2 – 16.1)
Moisture (% fw)	**11.08 ± 0.45 (9.01 – 13.30)**	**11.76 ± 0.45 (8.56 – 14.8)**	**(6.1 – 15.6)**
Total fat (%)	3.54 ± 0.09 (2.92 – 3.94)	3.59 ± 0.09 (2.88 – 4.13)	(1.7 – 4.3)
Protein (% dw)	12.43 ± 0.44 (10.30 – 14.77)	12.66 ± 0.44 (11.02 – 14.84)	(6.7 – 13.4)

dw=dry weight; fw=fresh weight.

a The mean of all values ± standard error of the mean.

b The range of sample values for commercial lines grown in 1998 (Sidhu *et al.* 1999)

5.1.2 Proximate analysis – European study

The statistical evaluation of the combined data from all sites showed that there were no statistically significant differences between line NK603 and the control line for the content of moisture, fat, protein, ash, carbohydrate, ADF and NDF in forage, and for the content of ash, moisture, ADF, NDF, carbohydrate and protein in grain. There was a statistically significant ($p<0.001$) difference between the two lines in the percentage of total fat in the grain, as shown in Table 4. As this difference was not observed in the forage from the same sample set, nor was it a consistent observation on a site-by-site basis, the result is considered to reflect random fluctuations generally observed when multiple compositional studies are undertaken at a range of different agricultural sites. Moreover, the range of values obtained for both the NK603 line and the control line were within the published literature range (Watson, 1982) for this component.

5.1.3 Amino acid analysis

Analysis of corn grain and forage included measurement of 18 essential amino acids, excluding glutamine and asparagine. In the U.S. study, with the exception of phenylalanine, none of the amino acid measurements for the grain showed a statistically significant difference between the transformed line NK603 and the non-transformed control. For the amino acid phenylalanine, in grain from NK603 assayed from all sites, the mean value was 5.34 ± 0.032 (% of total), whereas the mean value for phenylalanine in the non-transformed control over all sites was 5.26 ± 0.032 (% of total).

These values compare favourably to the commercial range for phenylalanine of 4.7 to 5.5 as represented by lines grown commercially in 1998, and the literature range of 2.9 to 5.7 (Watson, 1992).

The magnitude of the difference in the values for phenylalanine between the transformed line and its comparator is small, and is not of concern with respect to food safety. Moreover, both the lines are within the previously reported numerical ranges for this amino acid in commercialised corn grain.

Amino acids – European trials

Of the eighteen amino acids measured, small statistically significant differences were observed in six, including alanine, arginine, glutamic acid, histidine, lysine and methionine. The mean levels of some amino acids showed small increases (for example, alanine 1%; glutamic acid 2%) while for others the levels showed small decreases (for example, histidine 4%; arginine 6%). The results of the analysis of these combined data are presented in Table 4. As previously described for the proximate analysis results, the differences were not observed consistently across all sites in the study and therefore are not indicative of a general trend but rather reflect normal fluctuations in physiological parameters associated with plants grown at a variety of locations. Furthermore, when data from both the U.S. and European sites are compared, there is no pattern of significant changes observed across the statistical data.

Table 4: Summary of statistical results for the comparison of the grain from corn line NK603 and the non-transformed control (all European sites)

Component	Mean NK603	Mean Control	Mean Difference	Significance (p-value)	Mean Difference (% of control value)
Alanine (% total aa)	8.04	7.95	0.09	0.042	1.13
Arginine (% total aa)	4.00	4.27	-0.27	0.019	-6.32
Glutamic acid (% total aa)	19.93	19.40	0.53	0.009	2.73
Histidine (% total aa)	2.65	2.77	-0.12	0.003	-4.33
Lysine (% total aa)	2.71	2.83	-0.12	0.015	-4.24
Methionine (% total aa)	1.77	1.89	-0.12	0.031	-6.35
Total fat (% dry weight)	**4.16**	**3.6**	**0.56**	**<0.001**	**15.56**

5.1.4 Fatty acid content of grain

Corn oil is a processed fraction of the grain with important human food uses. The applicant provided detailed data together with a statistical evaluation relating to the fatty acids comprising corn oil. The data are presented in Table 5.

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.

The following fatty acid components are not listed in the table since the results of the analysis showed that >50% of values were below the limit of detection of the assay and hence were not used in the statistical analysis: 8:0 caprylic acid; 10:0 capric acid; 12:0 lauric acid; 14:0 myristic acid; 14:1 myristoleic acid; 15:0 pentadecanoic acid; 15:1 pentadecanoic acid; 16:1 palmitoleic acid; 17:0 heptadecanoic acid; 17:1 heptadecanoic acid; 18:3 gamma linolenic acid; 20:2 eicosadienoic acid; 20:3 eicosatrienoic acid; and 20:4 arachidonic acid.

Table 5: Summary of Fatty Acid analysis (% of total) of corn grain from all U.S. trial sites.

Component	NK603 Mean ± S.E. [a] (Range)	Control Mean ± S.E. [a] (Range)	Commercial[b] (Range)
16:0 Palmitic acid	9.16 ± 0.077 (8.67 – 9.57)	8.92 ± 0.077 (8.41 – 9.44)	(8.8 – 13.8)
18:0 Stearic acid	1.95 ± 0.028 (1.80 – 2.06)	1.86 ± 0.028 (1.67 – 1.98)	(1.4 – 2.6)
18:1 Oleic acid	22.46 ± 0.16 (21.37 – 23.12)	23.08 ± 0.16 (22.15 – 24.14)	(20.7 – 37.7)
18:2 Linoleic acid	64.49 ± 0.22 (63.79 – 65.80)	64.18 ± 0.22 (63.07 – 65.65)	(48.0 – 66.1)
18:3 Linolenic acid	1.10 ± 0.0096 (1.07 – 1.17)	1.11 ± 0.0096 (1.07 – 1.20)	(0.9 – 1.5)
20:0 Arachidic acid	0.37 ± 0.0057 (0.34 – 0.39)	0.37 ± 0.0057 (0.33 – 0.40)	(0.3 – 0.6)
20:1 Eicosenoic acid	0.29 ± 0.0062 (0.28 – 0.32)	0.30 ± 0.0062 (0.27 – 0.34)	(0.2 – 0.4)
22:0 Behenic acid	0.17 ± 0.0036 (0.14 – 0.19)	0.17 ± 0.0036 (0.14 – 0.19)	(0.1 – 0.3)

[a] The mean of all values ± standard error of the mean.
[b] The range of sample values for commercial lines grown in 1998 (Sidhu *et al.* 1999)

The results show that for the majority of fatty acids comprising corn oil, there was no difference between the results from the transformed NK603 and non-transformed lines. At some individual sites, there were statistically significant differences in the measurements of either stearic acid (18:0) or palmitic acid (16:0). Differences which were observed for only one or two of these site comparisons, and not observed across all of the trial site comparisons, do not represent a meaningful compositional difference between the test and control lines.

When data from all of the sites were analysed together, only stearic acid levels were found to be significantly different ($p<0.001$) between lines. However, neither stearic acid nor palmitic acid is a major component in corn oil. Although some differences were found with the statistical analysis, the magnitude of the difference between the comparators was small and both values are well within the reported ranges for other varieties of corn (see Table 4).

Fatty acids – European trials

The fatty acid compositional data for the grain show that there were no significant differences between corn line NK603 and the control that were consistently observed across a number of sites. Whereas in the U.S. study, stearic acid levels were increased approximately 4% (range 3.7 –5.1%), in the European study the stearic acid levels were decreased approximately 4% at one of the sites and were not significantly different at other sites used in the study.

Conclusion from fatty acid analyses

Overall, examination of the raw data from both the U.S. and European studies does not reveal differences in the fatty acid composition of the grain from the transformed and the non-transformed lines that are indicative of a systemic change. The data are explained by the known natural variation in composition due to a broad range of factors that influence plant growth and biochemistry.

5.1.4 Inorganic analysis

Measurements pertaining to inorganic components included the levels of nine minerals. The results of the mineral analysis are presented in Table 6. Sodium has been omitted as greater than 50% of the values were found to be below the limit of detection.

The statistical analysis showed that measurements for calcium, magnesium and phosphorus varied slightly between transformed and non-transformed lines at some sites, but the differences were not consistently observed across all sites. Overall, the data show that there were no statistically significant differences in mineral components of the NK603 corn and control lines.

5.1.5 Additional analysis

Vitamin E (tocopherol) occurs primarily in wheat seedlings, and has been isolated from wheat seedling oil. It is also present in lettuce, celery, cabbage, corn, palm oil, ground nuts, soybeans, castor oil and butter. Although there are numerous structural isomers of tocopherol, biologically, α- tocopherol is the most important member of the group.

The applicant provided measurements of the vitamin E content of the grain from transformed line NK603 corn and the non-transformed control, which are presented below in Table 7. The reference substance used for the assay was USP alpha tocopherol, 100%, lot number L1. The results show that the genetic modification in line NK603 did not result in any change to the naturally occurring low levels of vitamin E in corn.

Table 6: Summary of mineral analysis of corn grain from all trial sites in the study.

Component	NK603 Mean ± S.E. [a] (Range)	Control Mean ± S.E. [a] (Range)	Commercial[b] (Range)
Calcium (%)	0.0047 ± 0.00021 (0.0037 – 0.0056)	0.0044 ± 0.00021 (0.0033 – 0.0058)	(0.003 – 0.009)
Copper (mg/kg dw)	1.81 ± 0.090 (1.19 – 2.37)	1.92 ± 0.090 (1.50 – 2.33)	(0.9 – 2.8)
Iron (mg/kg dw)	22.69 ± 0.76 (19.08 – 25.94)	22.93 ± 0.76 (18.77 – 26.62)	(11 – 49)
Magnesium (%)	0.12 ± 0.0021 (0.11 – 0.13)	0.12 ± 0.0021 (0.11 – 0.13)	(0.08 – 0.2)
Manganese (mg/kg dw)	6.26 ± 0.32 (4.64 – 9.63)	6.25 ± 0.32 (4.96 – 8.83)	(2.6 – 7.8)
Phosphorus (%)	0.36 ± 0.0046 (0.32 – 0.39)	0.36 ± 0.0046 (0.32 – 0.39)	(0.24 – 0.43)
Potassium (%)	0.37 ± 0.0057 (0.35 – 0.39)	0.37 ± 0.0057 (0.34 – 0.41)	(0.29 – 0.53)
Zinc (mg/kg dw)	29.28 ± 0.88 (20.23 – 33.17)	29.66 ± 0.88 (23.47 – 33.26)	(15 – 33)

[a] The mean of all values ± standard error of the mean.
[b] The range of sample values for commercial lines grown in 1998 (Sidhu *et al.* 1999)

5.1.6 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). Phytic acid is also present in low amounts in corn, binding approximately 60-75% of the phosphorus in the form of phytate. Phytic acid levels in maize grain vary from 0.45 to 1.0% of dry matter (Monsanto, 1995; Watson, 1982).

Trypsin inhibitor activity is traditionally determined by enzymatic methods, but these methods are very dependent on the concentration of protein, non-protein inhibitors and other factors. The applicant compared the trypsin inhibitor activity of the transformed and non-transformed corn grain using a modified enzyme activity assay (limit of detection was 1.0 TIU/mg fresh weight of sample). In addition, data on the levels of phytic acid were provided.

The results presented in Table 7, show that the levels of anti-nutrient compounds, phytic acid and trypsin inhibitor, measured across all sites, in corn line NK603 are similar to the levels found in the untransformed control line.

Table 7: Summary of analysis (% of total) of corn grain from all trials.

Component	NK603 Mean ± S.E. [a] (Range)	Control Mean ± S.E. [a] (Range)	Commercial[b] (Range)
Phytic acid (%)	0.95 ± 0.028 (0.7 – 1.06)	0.97 ± 0.028 (0.81 – 1.21)	(0.5 – 1.3)
Trypsin inhibition (TIU/mg fw)	3.41 ± 0.27 (2.34 – 5.08)	2.91 ± 0.27 (1.39 – 5.14)	(3.4 – 7.18)
Vitamin E (mg/g dw)	0.0090 ± 0.00026 (0.0070 – 0.010)	0.0092 ± 0.00026 (0.0064 – 0.011)	(0.006 – 0.022)

[a] The mean of all values ± standard error of the mean.
[b] The range of sample values for commercial lines grown in 1998 (Sidhu *et al.* 1999)

5.2 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 reassurance that the food is nutritionally adequate. Such studies may be considered necessary where the compositional analysis indicates significant differences in a number of important components or nutrients or where there is concern that the bioavailability of key nutrients may be compromised by the nature of the genetic changes to the food.

In view of the compositional data available for corn line NK603 and the technical features of the genetic modification, animal studies were not considered essential to demonstrate the wholesomeness and nutritional adequacy of this food. Nevertheless, a feeding study using transformed and non-transformed corn grain was provided as additional supporting information and has been included in the safety assessment. Rapidly growing broiler chickens are sensitive to changes in nutrient quality in diets, and therefore serve as a useful model species to evaluate the wholesomeness of protein/amino acid sources.

Feeding study in Broiler Chickens

Study submitted:
George, B. *et al.*, 2001. Comparison of Broiler Performance When Fed Diets Containing Event NK603, Parental Line or Commercial Corn. Monsanto Study No. 2000-01-39-02.

This study compares the broiler performance and processing parameters of rapidly growing broiler chickens (*Gallus domesticus*) raised on a diet containing either corn event NK603, the non-transformed parental corn line (B73HTxLH82), or one of five commercially available reference corn lines, over approximately 43 days. Grain from the NK603 and parental lines was produced in field sites in Hawaii, while grain from the five reference lines was produced either in Hawaii or in other locations during the 1999/2000 growing season.

All diets were formulated to meet nutritional recommendations (National Research Council, 1994), based on individual nutrient analyses for the grain from each test line and control, and to align them with traditional broiler industry uses. From days 1-20, chickens were fed a starter diet containing approximately 55% w/w corn (crude protein ranging from 20.7% – 21.9%). From days 20-42, chickens were fed a grower/finisher diet containing approximately 60% w/w corn. These dietary corn concentrations are within the range used by commercial poultry growers in the United States. No growth promotants or other medications were added to the test diets which were provided *ad libitum.*

The birds, a high-yielding commercial strain (Ross x Ross 508), were one day of age at the beginning of the study, and were separated by gender and randomly assigned to treatments. For each treatment group, there were 100 birds (50 males and 50 females) in 10 pens (10 birds/pen), giving a total of 700 birds. During the course of the study, the birds were examined twice daily for general health, and any abnormal health symptoms were recorded. Any birds sacrificed were weighed, and any deaths were necropsied to determine the possible cause of death. As much as possible, environmental conditions simulated commercial conditions for raising broilers to market weight (around 2 kg) in approximately 42 days.

At study termination (day 43 for males, day 44 for females), carcass measurements were taken including those for fat pads which were collected from each bird and weighed. Meat quality assays on breast and thigh meat samples were subsequently conducted. Statistical analyses were performed on starting and final live weights, feed consumption, feed efficiency, chill weight, percent chill weight (for breast, wing, thigh and drum), as well as moisture, protein and fat for breast and thigh meat.

Results

The rate of chick mortality was at expected levels (average of 1.14% across groups) for commercial feeding trials and was randomly distributed across all treatments. All performance parameters measured were similar for all of the diets, including the NK603 corn, the non-transformed parental corn and commercial reference lines, as well as being comparable to published literature values for Ross x Ross broiler strains. In particular, live weight at day 0 and day 42, total feed intake and feed efficiency were similar across all treatments. Furthermore, no differences were observed in the percentage of moisture, protein and fat in breast meat or in the percentage of protein and fat in thigh meat across treatment diets. Finally, no differences were observed between the treatment groups in terms of wing weight measurements.

The results of the broiler feeding study show that there were no differences in parameters tested between birds fed a diet containing corn line NK603 and the non-transformed parental line (B73HT x LH82). In addition, when individual treatment comparisons were made, broiler chickens in general performed and had similar carcass yield and meat composition with diets containing NK603, the parental control, or five commercially available reference lines. The results support the conclusion that there are no differences between the non-transformed control and transformed corn line NK603 in terms of the ability to provide adequate nutrition to rapidly growing broiler chickens.

5.3 Conclusions from nutritional analyses

Comprehensive data from a range of compositional analyses conducted on grain from glyphosate treated corn line NK603 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, and inorganic mineral analysis.

The comparison of results and data from the test and control lines demonstrates that there are no compositional differences of biological significance between corn line NK603 and the non-transformed control in any of the components tested. Minor differences that were observed were not considered to be of concern with respect to food safety as the levels were well within published ranges that are normally expected of commercial corn varieties. Any variations between lines that were found following statistical evaluation of the data were small in magnitude, occurred at random across the trial sites, and were not indicative of a trend that might point to the existence of an unintended effect as a result of the genetic modification. Overall, as is expected with analyses of crop plants including corn, the compositional variation in a line across localities may be greater than the variation between different lines grown at the same site.

Finally, the feeding study using broiler chickens to test the nutritional adequacy of corn line NK603 compared to its parental control showed that there were no differences in the ability of the corn derived from either the test or control lines to support typical growth and nutritional well being.

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ATTACHMENT 4

DRAFT REGULATION IMPACT ASSESSMENT

Regulation Impact Assessment

The Authority is required, in the course of developing regulations suitable for adoption in Australia and New Zealand, to consider the impact of various options (including non-regulatory options) on all sectors of the community, including consumers, the food industry and governments in both countries. The regulatory impact assessment will identify and evaluate, though not be limited to, the disadvantages and advantages of the regulation, and its health, economic and social impacts.

Identification of affected parties

1. Governments in Australia and New Zealand

2. Consumers in Australia and New Zealand

3. Manufacturers, producers and importers of food products

Options

Option 1*–To prohibit the sale of food derived from corn line NK603*

GOVERNMENT Commonwealth, New Zealand Health Departments, State/Territory Health Departments	**Benefits** • no benefits were identified.	**Costs** • The governments of Australia and New Zealand may be challenged under the WTO to justify the need for more stringent restrictions than apply internationally. • A prohibition on food derived from corn line NK603 in Australia and New Zealand could result in retaliatory trade measures from other countries. • There may be technical problems for AQIS in enforcing such a prohibition at the import barrier.
INDUSTRY Manufacturers, producers and importers of food products	**Benefits** • Some companies may benefit from being able to exploit niche markets for non-GM products overseas.	**Costs** • Food manufacturers and producers will be unable to use the processed food fractions from foods derived from corn line NK603 thus requiring segregation of total corn harvest or the switch to non-GM ingredients and the reformulation of many processed food products. The cost to manufacturers of going non-GM has been estimated to be $A 207m in Australia and $NZ 37m in New Zealand[9]. This is equivalent to 0.51% of turnover in Australia and 0.19% in New Zealand.

[9] Report on the costs of labelling genetically modified foods (2000)

CONSUMERS	**Benefits** • For those consumers who perceive that GM foods in general are unsafe, not permitting food derived from corn line NK603 would be an advantage.	**Costs** • Could lead to short-term decreased availability of certain food products. • Increased costs to consumers would be likely because manufacturers and producers may have to source non-GM ingredients or demand identification of crop variety and subsequent segregation of the total harvest..

Option 2*– To permit the sale of food produced using gene technology*

GOVERNMENT Commonwealth, New Zealand Health Departments, State/Territory Health Departments	**Benefits** • Economic benefits flow to government from increased innovation and competitiveness in the food industry.	**Costs** • Minor costs associated with amending the *Food Standards Code.*
INDUSTRY Manufacturers, producers and importers of food products	**Benefits** • **F**ood producers and manufacturers will be able to capitalise on the latest technology. • The importation of manufactured food products from overseas markets will not be impeded where, for example, there is no restriction on the use of food produced using gene technology.	**Costs** • There may be discrimination against Australian and New Zealand food products in overseas markets that have a preference for some non-GM foods (e.g., Japan and the European Union).
CONSUMERS	**Benefits** • Consumers may have access to a greater range of food products.	**Costs** • Those consumers who wish to avoid GM food may experience restricted choice in food products. • Those consumers who wish to avoid GM food may have to pay more for non-GM food.

Conclusion of the regulatory impact assessment

Consideration of the regulatory impact for foods produced using gene technology concludes that the benefits of permitting foods produced using gene technology primarily accrue to the food industry and to government, with potentially a small benefit to consumers. These advantages are considered to outweigh the disadvantages to all sectors, provided the safety assessment does not identify any public health and safety concerns.

ATTACHMENT 5

WORLD TRADE ORGANIZATION AGREEMENTS

With the completion of the Uruguay Round of trade negotiations, the World Trade Organization (WTO) was created on 1 January 1995 to provide a forum for facilitating international trade.

The WTO does not engage in any standard-setting activities but is concerned with ensuring that standards and procedures for assessment of and conformity with standards do not create unnecessary obstacles to international trade.

Two agreements which comprise part of the WTO treaty are particularly important for trade in food. They are the;

- Agreement on the Application of Sanitary and Phytosanitary Measures (SPS); and
- Agreement on Technical Barriers to Trade (TBT).

These agreements strongly encourage the use, where appropriate, of international standards, guidelines and recommendations, such as those established by Codex (in relation to composition, labelling, food additives, veterinary drug and pesticide residues, contaminants, methods of analysis and sampling) and the code and guidelines on hygienic practice.

Both Australia and New Zealand are members of the World Trade Organization (WTO) and signatories to the agreements on the Application of Sanitary and Phytosanitary Measures (SPS agreement) and on Technical Barriers to Trade (TBT agreement). Within Australia, the Council of Australian Governments (COAG) has put in place a Memorandum of Understanding binding all States and Territories to the agreements.

The WTO agreements are predicated on a set of underlying principles that standards and other regulatory measures should be:

- based on sound scientific principles;
- developed using consistent risk assessment practices;
- transparent;
- no more trade-restrictive than necessary to achieve a legitimate objective;
- recognise the equivalence of similar measures in other countries; and
- not used as arbitrary barriers to trade.

As members of the WTO both Australia and New Zealand have an obligation to notify the WTO of changes to food standards to enable other member countries of the WTO to make comment. Notification is required in the case of any new or changed standards which may have a significant trade effect and which depart from the relevant international standard (or where no international standard exists). Matters raised in this proposal may be notified to the WTO as either SPS notifications or TBT notifications, or both.

SPS Notifications

These are primarily health related, and refer to any sanitary and phytosanitary measure applied:

- to protect animal or plant life from risks arising from the entry, establishment or spread of pests, diseases or disease carrying organisms;

- to protect human or animal life or health from risks arising from additives, contaminants, toxins or disease-carrying organisms in foods, beverages or foodstuffs;

- to protect human life or health from risks arising from diseases carried by animals, plants or products thereof, or from the entry, establishment or spread of pests; and

- to prevent or limit other damage from the entry, establishment or spread of pests.

The Agreement on the Application of Sanitary or Phytosanitary Measures relates to any sanitary or phytosanitary measure applied to protect animal, plant or human life or health which may directly or indirectly affect international trade. Whether the SPS measure is in the form of a law or mandatory regulation, an advisory guideline, a code of practice or a requirement, it is the purpose of the measure that is important - not its regulatory status. Each WTO member country is entitled to apply SPS measures that are more stringent than the international standards in order to protect the health of its population. In the interests of transparency, each instance of such non-alignment which could result in an impediment to trade must be identified and justified and the documentation of that justification must be readily available

Each member country is also required to apply its methods of risk assessment and management consistently so arrangements under the SPS Agreement do not generate what may really be technical barriers to trade

Under the SPS Agreement, an exporting country can have resort to the WTO's dispute settlement procedures with respect to such a non-alignment. These arrangements mean there is potential for a code of practice to introduce an SPS measure that may bring about non-alignment with international requirements. Such non-alignment would need to be justified scientifically on the grounds that it is necessary to protect human, animal or plant life or health.

TBT Notifications

A technical barrier to trade arises when a mandatory requirement in a country's food regulatory system does not align with the international standard and it is more trade restrictive than is necessary to fulfil a legitimate objective. However, it can be acceptable for a country to have a more stringent requirement than that set internationally for reasons including:

- Maintaining national security;
- Preventing deceptive practices; and
- Protecting human health or safety.

Instances of non-alignment with international standards which could result in trade barriers must be identified and, if questioned, justified. Voluntary codes of practice are not expected to generate technical barriers to trade except where compliance with a code of practice or some aspect of a code of practice is expected. Consequently, it is possible for a voluntary code of practice to be viewed by the WTO as mandatory and subject to all the notification and other provisions applying to mandatory regulations.

The Agreement on Technical Barrier to Trade relates to requirements covering product characteristics or their related processes and production methods. TBT covers measures that are not SPS, such as requirements relating to terminology, symbols, packaging, marking, labelling, food composition and processing methods.

ATTACHMENT 6

SUMMARY OF FIRST ROUND PUBLIC SUBMISSIONS

1. National Council of Women of Australia

- Oppose the approval of corn line NK603, stating that the purpose of the new line is unclear when the previously approved (glyphosate-tolerant) corn line GA21 (Application A362) is already in existence.
- State that under the revised labelling requirements, all products containing this food will not be labelled, and therefore consumers will be denied a choice.
- Indicate that more detailed comment on the safety evaluation is deferred until the next step in the process when the Draft Risk Analysis Report is released.

2. Food Technology Association of Victoria Inc.

- Supports the approval of corn line NK603.

3. Claire Bleakley (NZ)

- Opposes approval of this application until rigorous and independent studies have been carried out over the next 10 years to assess safety.
- States that recent publications in *Science* indicate that environmental risks are very complex to assess.
- Claims that not enough peer-reviewed animal feeding studies are conducted.
- Provides information on Roundup Ready soybeans obtained from the internet which claims that the US FDA fails to adequately assess GM foods before they are permitted on the market.
- Considers that information obtained over the internet proves that not enough is known about the process of genetic modification of the food supply.
- States that trade obligations compromise safety assessment processes.
- Maximum residue limits for the new GM foods are not listed in the New Zealand Food Regulations.
- Considers that the outbreak /epidemic of *E. coli* and *Salmonella* diseases is inexplicable except through the advent of GM technology.

4. Canberra Consumer

- Suggests that ANZFA's assessment should consider pleiotropic effects as part of the safety assessment process.
- States that differences in the levels of some essential amino acids were reported for other GM corns which could affect the nutritional value and these issues should be noted in the feeding studies using any new GM variety.

5. Safe Food Campaign (NZ)

- Strongly opposed to the approval of corn line NK603 because of the lack of long term health testing and the assumption that the herbicide residues will increase.
- State that approval would have broader social implications for people who want to avoid the consumption of food derived from corn line NK603.
- Regards the concept of *substantial equivalence* as flawed and calls for the type of rigorous toxicological testing that is applied to pharmaceuticals.
- Disagrees that food manufacturers will be negatively affected if corn line NK603 were not approved because consumer demand for non-GM and organic food is increasing.

- Any permission to use corn line NK603 would disadvantage the health of lower income earners who will buy the cheapest product on the market.
- Considers that there is an urgent need to monitor the health consequences of GM foods already on the market in New Zealand but acknowledges that this would be very difficult to do, especially when GM and non-GM versions are co-mingled.
- Although supportive of the clear labelling of all foods, fears that cross-pollination may compromise the effectiveness of labelling.
- States that there are scientists who claim that they would not consume produce from cows fed on GM corn, because of the unpredictable and unstable nature of GM technology creating the risk of unintended and unforeseen side effects.
- Claims that GM foods could create new allergies by exposing people to thousands of new proteins whose allergenic status is unknown. For example, proteins from leeches, waxmoths, mice, African clawed toad etc, are being used.
- No decisions on the approvals of any GM food should occur until the findings of the New Zealand Royal Commission on Genetic Modification have been released.
- Offers to make an oral submission on this application.

6. R. A. Randell (NZ)

- Opposed to the approval of corn line NK603 because considers that safety has not been adequately established.

ATTACHMENT 7

GENERAL ISSUES RAISED IN PUBLIC COMMENTS

The majority of submissions received in response to the Section 14 Gazette Notice, express general views against the use of gene technology and assert that food produced using this technology is unsafe for human consumption. A number of general issues were raised in these submissions that are addressed below.

1. *The safety of genetically modified foods for human consumption*

A majority of submitters raised the issue of public health and safety in relation to food produced using gene technology. In particular, it was stated that there has been inadequate testing of genetically modified foods, that there is limited knowledge concerning the risks associated with the technology and that there may be potential long–term risks associated with the consumption of such foods.

- *Evaluation*

It is a reasonable expectation of the community that foods offered for sale are safe and wholesome. In this context, *safe* means that there is a reasonable certainty of no harm. As with other aspects of human activity, the absolute safety of food consumption cannot be guaranteed. Conventionally produced foods, while having a long history of safe use, are associated with human disease and carry a level of risk which must be balanced against the health benefits of a nutritious and varied diet.

Because the use of gene technology in food production is relatively new, and a long history of safe use of these foods has yet to be established, it is appropriate that a cautious approach is taken to the introduction of these foods onto the market. The purpose of the pre–market assessment of a food produced using gene technology under Standard A18/Standard 1.5.2 is to establish that the new food is at least as safe as the existing food. The comprehensive nature of the scientific safety assessment, undertaken on a case-by-case basis, for each new modification is reflective of this cautious approach.

The safety assessment focuses on the new gene product(s), including intentional and unintentional effects of the genetic modification, its properties including potential allergenicity, toxicity, compositional differences in the food and it's history of use as a food or food product.

Foods produced using gene technology are assessed in part by a comparison with commonly consumed foods that are already regarded as safe. This concept has been adopted by both the World Health Organisation (WHO)/Food and Agriculture Organisation (FAO) and the Organisation for Economic Cooperation and Development (OECD). The Authority has developed detailed procedures for the safety assessment of foods produced using gene technology that are constantly under review to ensure that the process reflects both recent scientific and regulatory developments and are consistent with protocols developed internationally.

2. *The need for long-term feeding studies*

A number of submissions were concerned about the lack of long-term toxicity studies on genetically modified foods.

- *Evaluation*

Animal studies are a major element in the safety assessment of many compounds, including pesticides, pharmaceuticals, industrial chemicals and food additives. In most cases, the test substance is well characterised, of known purity and of no nutritional value, and human exposure is generally low. It is therefore relatively straightforward to feed such compounds to laboratory animals at a range of doses (some several orders of magnitude above expected human exposure levels) in order to identify any potential adverse effects. Establishing a dose-response relationship is a pivotal step in toxicological testing. By determining the level of exposure at which no adverse effects occur, a safe level of exposure for humans can be established which includes appropriate safety factors.

By contrast, foods are complex mixtures of compounds characterised by wide variations in composition and nutritional value. Due to their bulk, they can usually be fed to animals only at low multiples of the amounts that might be present in the human diet. Therefore, in most cases, it is not possible to conduct dose-response experiments for foods in the same way that these experiments are conducted for chemicals. In addition, a key factor to be considered in conducting animal feeding studies is the need to maintain the nutritional value and balance of the diet. A diet that consists entirely of a single food is poorly balanced and will compromise the interpretation of the study, since the effects observed will confound and usually override any other small adverse effect which may be related to a component or components of the food being tested. Identifying any potentially adverse effects and relating these to an individual component or characteristic of a food can, therefore, be extremely difficult. Another consideration in determining the need for animal studies is whether it is appropriate from an ethical standpoint to subject experimental animals to such a study if it is unlikely to produce meaningful information.

If there is a need to examine the safety of a newly-expressed protein in a genetically-modified food, it is more appropriate to examine the safety of this protein alone in an animal study rather than when it is part of a whole food. For newly-expressed proteins in genetically-modified foods, the acute toxicity is normally examined in experimental animals. In some cases, studies up to 14 days have also been performed. These can provide additional reassurance that the proteins will have no adverse effects in humans when consumed as part of a food.

While animal experiments using a single new protein can provide more meaningful information than experiments on the whole food, additional reassurance regarding the safety of newly-expressed protein can be obtained by examining the digestibility of the new protein in laboratory conducted *in vitro* assays using conditions which simulate the human gastric system.

3. *Substantial equivalence*

A number of submitters express concern regarding the use of the concept of substantial equivalence as part of the assessment process. Some reject the premise of substantial equivalence on the grounds that differences at the DNA level make foods substantially different.

- *Evaluation*

Substantial equivalence embodies the concept that, as part of the safety assessment of a genetically modified food, a comparison can be made in relation to the characteristics and properties between the new food and traditionally-produced food. This can include physical characteristics and compositional factors, as well as an examination of the levels of naturally occurring allergens, toxins and anti-nutrients.

This allows the safety assessment to focus on any significant differences between the genetically modified food and its conventionally produced counterpart. Genotypic differences (i.e. differences at the DNA level) are not normally considered in a determination of substantial equivalence, if that difference does not significantly change the characteristics for composition of the new food relative to the conventional food. This is partly because differences at the DNA level occur with every breeding event and often arise also as a result of certain environmental factors.

The concept of substantial equivalence allows for an evaluation of the important constituents of a new food in a systematic manner while recognizing that there is general acceptance that normally consumed food produced by conventional methods is regarded by the community as safe. It is important to note that, although a genetically modified food may be found to be different in composition to the traditional food, this in itself does not necessarily mean that the food is unsafe or nutritionally inadequate. Each food needs to be evaluated on an individual basis with regard to the significance of any changes in relation to its composition or to its properties.

The concept of *substantial equivalence* was first espoused by a 1991 Joint Consultation of the Food and Agricultural Organisation (FAO) and the World Health Organisation (WHO) where it was noted that the '*comparison of a final product with one having an acceptable standard of safety provides an important element of safety assessment*'. Since this time, the concept has been integrated into safety assessment procedures used by regulatory authorities worldwide. It has thus been in use for approximately ten years and has been an integral part of the safety assessment of some 40 products.

Although the concept of *substantial equivalence* has attracted criticism, it remains as the most appropriate mechanism for assessing the nutritional and food safety implications of foods produced using gene technology. It is generally agreed also that continual review of the concept, in response to the criticism, provides a useful stimulus to ensure that safety assessment procedures are kept at the forefront of scientific knowledge (Nick Tomlinson, Food Standards Agency, United Kingdom: Joint FAO/WHO Expert Consultation on Foods Derived from Biotechnology, Geneva, 2000).

4. *The nutritional value of food produced using gene technology*

A small number of submitters express concern that the genetic alteration of food decreases its nutritional value.

- *Evaluation*

The assessment of food produced using gene technology by ANZFA entails an exhaustive evaluation of analytical data on any intentional or unintentional compositional changes to the food. This assessment encompasses the major constituents of the food (fat, protein, carbohydrate, fibre, ash and moisture) as well as the key nutrients (amino acids, vitamins, fatty acids). There is no evidence to suggest that genetic modification *per se* reduces the nutritional value of food.

In the future, genetic modification may be used intentionally to improve the nutritional value of food. In this regard, GM foods may be able to assist in addressing the general nutritional needs of the community and also specific dietary needs of sub-populations.

5. *Potential toxins and allergens*

Some submitters express concerns about the risks of the introduction of new toxins or allergens.

- *Evaluation*

This issue is considered in detail as part of the safety assessment conducted on each new genetic modification applied to a food or commodity crop. New toxins or allergens may be introduced into food by either gene technology or by traditional breeding techniques, or by altered production processes. It is also possible to use these techniques to develop foods specifically where such compounds are significantly reduced or eliminated. One advantage of gene technology, in comparison with these other methods, is that any transferred genes are well characterised and defined, thus the possibility of developing a food with a new toxic or allergenic compound is likely to be reduced.

6. *Antibiotic resistance*

Some submitters raise concerns about an increase in antibiotic resistance resulting from the use of gene technology. Some consider that it would be reassuring if independent biomedical advice were available to inform the public that the use of antibiotic resistance markers does not pose a risk to the future use of antibiotics in the management of human disease.

- *Evaluation*

The human health considerations in relation to the potential for the development of antibiotic resistance depend on the nature of the novel genes and must be assessed on a case-by-case basis. This issue arises because of the use of antibiotic resistance marker genes in the generation of some genetically modified plants. Antibiotic resistance genes can be linked to the gene of interest, to enable the initial selection of the engineered cells in the laboratory. Those cells that contain the antibiotic resistance marker gene, and hence the gene of interest, will be able to grow in the presence of the corresponding antibiotic.

Those cells that failed the transformation process are eliminated during the selection procedure. Where transformed cells can be selected by other methods, there is no absolute requirement for the presence of the antibiotic resistance marker gene.

Concern has arisen that ingestion of food containing copies of antibiotic resistance genes could facilitate the transfer of the gene to bacteria inhabiting the gut of animals and humans. It is argued that these genes may then be transferred to disease causing bacteria and that this would compromise the therapeutic use of these antibiotics.

In 1993, the World Health Organisation Food Safety Unit considered this issue at a Workshop on the health aspects of marker genes in genetically modified plants. It was concluded at that Workshop that the potential for such gene transfers is effectively zero, given the complexity of the steps required. Since this time, several separate expert panels (Report to the Nordic Council, Copenhagen 1996; Advisory Committee on Novel Foods and Processes, UK 1994, 1996; The Royal Society, UK 1998) and numerous scientific papers published in peer reviewed journals have also considered the available evidence on this issue. It is generally agreed that the presence and subsequent transfer of an intact functional gene from transgenic food to micro-organisms in the human intestine is an extremely unlikely event. Furthermore, if this were to occur, bacteria would not normally retain the resistance genes unless there was an environment for positive selection. The majority of these genes provide for resistance to antibiotics whose use is confined to the laboratory and are not considered to be of major therapeutic use in humans.

Antibiotic resistant bacteria are naturally occurring, ubiquitous and normally inhabit the gut of animals and humans. There is a general consensus that the transfer of antibiotic resistance genes is much more likely to arise from this source and from associated medical practices, rather than from ingested genetically modified food. Even so, at the recent OECD Conference (GM Food Safety: Facts, Uncertainties, and Assessment) held in Edinburgh on 28 February – 1 March 2000, there was general consensus that the continued use of antibiotic marker genes in GM food crops is unnecessary given the existence of adequate alternatives, and should be phased out.

7. Transfer of novel genes

Some submitters have expressed concern that the transfer of any novel gene may be a health concern.

- *Evaluation*

It is extremely unlikely that novel genetic material will transfer from GM foods to bacteria in the human digestive tract because of the number of complex and unlikely steps that would need to take place consecutively. It is equally unlikely that novel genetic material will transfer from GM foods 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 GM 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.

8. *Viral recombination*

Some submitters express concern about the long term effects of transferring viral sequences to plants.

- *Evaluation*

This is an issue that is commonly raised because some of the genes that are transferred to plants use a plant virus promoter. Promoters are controlling DNA sequences which act like a switch and enable the transferred genes to be expressed (i.e. to give rise to a protein product) in a plant cell. The routine use of these viral promoters is often confused with research which has shown that plant virus genes, which have been transferred into plants to render them virus–resistant, may recombine with related plant viruses that subsequently infect the plant, creating new viral variants. This research demonstrates that there may be a greater risk to the environment if viral genes are transferred to plants because it may lead to the generation of new plant virus variants capable of infecting a broader range of plants. This is a matter that will be addressed by the Genetic Manipulation Advisory Committee (GMAC) on a case–by–case basis when it assesses such plants.

However, the presence of plant viruses, plant virus genes or plant virus segments in food is not considered to pose any greater risk to human health as plant viruses are ubiquitous in nature and are commonly found in food eaten by animals and humans. Plant viruses are also biologically incapable of naturally infecting human or animal cells.

9. *Labelling of foods produced using gene technology*

A majority of submissions focus on this issue. Specifically, the submissions call for comprehensive labelling of foods produced using gene technology, regardless of whether they are substantially equivalent to conventional foods. The submitters base their demands for full labelling on the presumption that all foods produced using gene technology are unsafe, even where no novel genes are present, and on consumer "right to know" arguments. It is stated that full labelling is the only means of identification of foods produced using gene technology available to consumers.

- *Evaluation*

In response to consumer sentiment on this issue, on 28 July 2000, Health Ministers (from New Zealand, the Commonwealth, States and Territories of Australia) agreed to new labelling rules for genetically modified foods. Amendments to the Standard were subsequently confirmed by the Ministerial Council on 24 November 2000 and finally gazetted on 7 December 2000. The amended Standard A18 (Volume 1) is now also known as Standard 1.5.2 in the joint Australia New Zealand Food Standards Code (Volume 2). To allow adequate time for compliance to the new provisions of the Standard, it will come into effect on 7 December 2001, twelve months after the date of gazettal.

The new Standard requires the labelling of food and food ingredients where novel DNA and/or protein is present in the final food and where the food has altered characteristics.

Exempt from these requirements are:

- highly refined food, where the effect of the refining process is to remove novel genetic material and/or protein;
- processing aids and food additives, except where novel genetic material and/or protein is present in the final food;
- flavours which are present in a concentration less than or equal to 0.1 per cent in the final food; and
- food prepared at point of sale (e.g. restaurants, takeaway food outlets).

In addition, the new Standard allows for a maximum of 1 per cent of unintended presence of genetically modified product, as ascertained by laboratory testing, before labelling would be required. The comprehensive provisions of the new Standard represent the culmination of extensive consultation between government, consumers and the food industry to ensure practical and relevant information is available to all in relation to the sale of genetically modified foods.

A User Guide has been prepared by the Authority under direction of the Ministerial Council, to assist with compliance with the amended labelling provisions of the Standard. A copy of the guide is available on the ANZFA website (www.anzfa.gov.au).

10. The need for post marketing surveillance of genetically modified foods

A number of submitters have commented on the need for post-market surveillance of genetically modified food consumption.

- *Evaluation*

Surveillance of potential adverse or beneficial effects of GM foods is seen by many as a logical follow-up to the initial scientific risk assessment. Nevertheless, it is recognised that there are limitations to the application of epidemiology studies, particularly in relation to food components. A key requirement for post-market surveillance systems is that a clear hypothesis be identified for testing. Establishing a system for the surveillance of potential health effects of exposure to novel foods requires monitoring of the consumption patterns of novel foods in the population, and health effects in both “exposed” and “non-exposed” individuals/populations, so that risk estimates can be derived. For any such monitoring system to be useful, there needs to be a range of exposures, otherwise, any variation in health outcome would be unexplainable by that exposure. Variations in exposure could be apparent over time (temporal trends), space (geographical trends) or both.

Availability of robust data on consumption of the foods in question is vital in order to establish a surveillance system. The other side of the equation is the need for access to data on population health outcomes. Such a system could also be used to identify potential positive health outcomes, such as improved nutritional status or lower cholesterol levels. The availability of linked basic data (e.g. date of birth, sex, geographical location), and the ability to correlate with demographic data, could potentially offer the means of establishing links with food consumption.

The possibility of setting up a post-market health surveillance system for novel foods, including GM foods, has been examined by the UK's Advisory Committee on Novel Foods and Processes (ACNFP). Recognising the many difficulties involved in developing such a system, an initial feasibility study to look at the available data and its usefulness has been proposed. Work is currently being commissioned; when completed in 18 months, it will be subject to peer review. If such a feasibility study suggests that post-market surveillance is practical, methods and details concerning data collection will be determined in the UK, but common strategies might be able to be harmonised internationally in order to minimise the use of resources while maximising the reliability of the final results. This is an area that ANZFA will be monitoring closely, along with international regulatory bodies such as the OECD Taskforce for the Safety of Novel Foods and Feeds.

11. ***Public consultation and information about gene technology***

A number of submitters were concerned that the public has not been properly consulted or informed by government or ANZFA on the introduction of foods produced using gene technology. Some submitters urged to undertake wider consultation with all affected parties including growers, the food industry and consumers before these food commodities are introduced, and to ensure that adequate consultation is undertaken as part of its assessment process.

- *Evaluation*

The issue of gene technology and its use in food has been under consideration in Australia since 1992. The Agreement between the Governments of Australia and New Zealand for a joint food standard setting system, however, did not occur until 1995, and the New Zealand community therefore had not been consulted on this matter by the Authority until after that time. Consequently, the proposed standard (the current Standard A18) underwent only one round of public comment in New Zealand at which time significant objections were raised by the New Zealand community to the use of gene technology in food production. Many New Zealand consumers, both in these submissions, and in previous submissions to the Authority, have expressed the view that there has been insufficient consultation and a consistent lack of information about gene technology.

Although Standard A18 came into force in May 1999, the public have a continuous and ongoing opportunity to provide comment in relation to applications under the standard. ANZFA's statutory process for all applications to amend the *Food Standards Code* normally involves two rounds of public comment. Furthermore, all the documentation (except for commercial in confidence information) relating to these applications is available in the public domain, including the safety assessment reports. There is ample evidence that the provision of such information by ANZFA has already significantly stimulated public debate on this matter.

In addition, other government departments including the Environmental Risk Management Authority (ERMA) are potential sources of information about gene technology available to consumers in New Zealand. ERMA is a statutory authority set up by the New Zealand Government to administer the *Hazardous Substances and New Organisms (HSNO) Act 1996*, and has responsibility for assessing the risks to the environment from genetically modified organisms. This body has been assessing applications for the approval of genetically modified organisms since July 1998 and this has involved a number of public meetings.

In response to the concerns raised in public submissions with regard to gene technology and GM foods, ANZFA has prepared a public discussion paper on the safety assessment process for GM foods[10], available at no charge on request. Since completion, this document has been widely distributed and may assist in addressing some of the concerns raised by the public. Other government and industry bodies are also addressing the broader concerns in relation to gene technology.

12. *Maori beliefs and values*

Some New Zealand submitters stated that Maori people find genetic engineering in conflict with their beliefs and values and that, out of respect to Maori, no genetically modified foods should be allowed into New Zealand until a wider discussion, both within Maori and non–Maori, is held.

- *Evaluation*

This issue was also raised during consideration of the proposal for the establishment of Standard A18. At that time, it was stated that the likely implications for Maori regarding genetically modified organisms surround the issues of the rights of Maori to the genetic material from flora and fauna indigenous to New Zealand and the release into the environment of genetically modified organisms. The *HSNO Act 1996* requires that these matters be considered by ERMA.

13. *Environmental concerns and the broader regulatory framework*

A number of submitters have raised concerns that genetically modified crops may pose a risk to the environment.

- *Evaluation*

These issues are considered as part of the comprehensive assessment processes of the Office of the Gene Technology Regulator (OGTR) in Australia, and the Environmental Risk Management Authority (ERMA) in New Zealand. Since June 2001, OGTR regulates all GMOs and any 'gap' products (i.e. products for which no other regulator has responsibility).

The Australia New Zealand Food Authority (ANZFA) does not have the mandate to assess matters relating to environmental risks resulting from the release of foods produced using gene technology into the environment. However, links exist between ANZFA and these other regulatory agencies in both Australia and New Zealand, and a large degree of information sharing occurs.

In Australia, the current regulatory system includes a number of other agencies with a legal remit to cover some aspects of GM products (such as imports, food, agricultural and veterinary chemicals):

- the Australia New Zealand Food Authority (ANZFA)
- the Therapeutic Goods Administration (TGA)

[10] Gm foods and the consumer – ANZFA Occasional Paper Series No.1, Australia New Zealand Food Authority, June 2000.

- the National Registration Authority for Agricultural and Veterinary Chemicals (NRA)
- the National Industrial Chemicals Notification and Assessment Scheme (NICNAS)
- the Australian Quarantine and Inspection Service (AQIS).

All GM foods continue to be assessed and regulated by ANZFA under the direction of Commonwealth, State and Territories Health Ministers and the New Zealand Health Minister, sitting as the Australia New Zealand Food Standards Council (ANZFSC). However, an interface between ANZFA and OGTR has been established through amendments to the ANZFA Act arising from the Gene Technology Bill 2000. These amendments to the ANZFA Act require the Authority to advise OGTR of recommendations to ANZFSC regarding the standard for foods produced using gene technology (Standard A18/1.5.2).

Similarly, in New Zealand various other government departments and agencies play their role in the regulatory process:

- the Ministry of Agriculture and Fisheries (MAF)
- the Ministry of Health (MoH)
- the Ministry of Research, Science and Technology (MoRST)

14. Maximum residue levels of agriculture/veterinary chemicals

A number of submitters have raised concerns that residues of agricultural and veterinary chemicals in genetically modified (e.g. herbicide tolerant) crops may pose a health risk.

- *Response*

Residues of these chemicals can only legally be present if the chemical has been registered for use in Australia and/or New Zealand, and it has been demonstrated that the residue at specified levels does not lead to adverse health impacts. The concentration of a chemical residue that may be present in a food is regulated through maximum residue limits (MRLs). The MRL is the highest residue concentration that is legally permitted in the food. Food products have to meet the MRL, whether or not they are derived from genetically modified organisms. The MRL does not indicate the chemical residue level that is always present in a food, but it does indicate the highest residue level that could result from the registered conditions of use.

It is important to note that MRLs are not direct public health and safety limits but rather, are primarily indicators of appropriate chemical usage. MRLs are always set at levels lower than, and normally very much lower than, the health and safety limits. The MRL is determined following a comprehensive evaluation of scientific studies on chemistry, metabolism, analytical methods and residue levels. In Australia, the National Registration Authority (NRA) applies to ANZFA to amend the MRLs in the Food Standards Code and the application is considered by ANZFA through its legislated decision making processes. In New Zealand MRLs are set by the Ministry of Health, generally following a request from, and in collaboration with, the Ministry of Agriculture and Forestry. Only following demonstration that the use of agricultural and veterinary chemicals will not result in unsafe residues will the MRL enter into food law, through its inclusion in either the Food Standards Code in Australia, or the New Zealand Mandatory Food Standard 1999 (Maximum Residue Limits of Agricultural Compounds).