EXPLANATORY STATEMENT

APPLICATION A592

FOOD DERIVED FROM GLYPHOSATE-TOLERANT SOYBEAN MON89788

For Information on matters relating to this Assessment Report or the assessment process generally, please refer to http://www.foodstandards.gov.au/standardsdevelopment/

Executive Summary

An Application has been received from Monsanto Australia Limited to amend the *Australia New Zealand Food Standards Code* (the Code) to approve food derived from genetically modified (GM) herbicide-tolerant soybean MON 89788. Standard 1.5.2 – Food produced using Gene Technology requires that GM foods undergo a pre-market safety assessment before they may be sold in Australia and New Zealand.

Soybean MON 89788 has been genetically modified to be tolerant to the herbicide glyphosate. FSANZ has undertaken a safety assessment of glyphosate-tolerant soybean MON 89788. If approved, food derived from glyphosate-tolerant soybean MON 89788 may enter Australia and New Zealand as imported products. It is not intended that MON 89788 be cultivated in Australia or New Zealand

The herbicide tolerance trait introduced into glyphosate-tolerant soybean MON 89788 is conferred by expression in the plant of an enzyme, CP4 EPSPS, derived from a common soil bacterium. No marker genes are present in glyphosate-tolerant soybean MON 89788.

Safety assessment

FSANZ has completed a comprehensive safety assessment of food derived from glyphosate-tolerant soybean MON 89788, as required under Standard 1.5.2. The assessment included consideration of (i) the genetic modification to the plant; (ii) the potential toxicity and allergenicity of the novel protein; and (iii) the composition of glyphosate-tolerant soybean MON 89788 compared with that of conventional soybean.

The assessment of this Application identified no public health and safety concerns. On the basis of the available evidence, including detailed studies provided by the Applicant, food derived from glyphosate-tolerant MON 89788 is considered as safe and wholesome as food derived from commercial soybean varieties.

Labelling

Foods derived from glyphosate-tolerant soybean MON 89788 will be required to be labelled as genetically modified if novel DNA and/or novel protein is present in the final food. Studies conducted by the Applicant show that the novel protein is present in the unprocessed grain. Highly refined products, such as soybean oil, will not require labelling if they do not contain novel protein or DNA.

Labelling addresses the requirement of paragraph 18(1)(b) of the *Food Standards Australia New Zealand Act 1991*; provision of adequate information relating to food to enable consumers to make informed choices.

Impact of regulatory options

Two regulatory options were considered in the assessment: (1) no approval; or (2) approval of food derived from glyphosate-tolerant soybean MON 89788 based on the conclusions of the safety assessment.

Following analysis of the potential costs and benefits of each option on affected parties (consumers, the food industry and government), approval of this application is the preferred option as the potential benefits to all sectors outweigh the costs associated with the approval.

Purpose

The Applicant seeks amendment to Standard 1.5.2 – Food produced using Gene Technology, to include food derived from glyphosate-tolerant soybean MON 89788 in the Table to clause 2.

Decision

Amend Standard 1.5.2 – Food produced using Gene Technology, to include food derived from glyphosate-tolerant soybean MON 89788 in the Table to clause 2.

Reasons for Decision

An amendment to the Code approving food derived from glyphosate-tolerant soybean MON 89788 in Australia and New Zealand is approved on the basis of the available scientific evidence, for the following reasons:

- the safety assessment did not identify any public health and safety concerns associated with the genetic modification used to produce glyphosate-tolerant soybean MON 89788;

- food derived from glyphosate-tolerant soybean MON 89788 is equivalent to food from other commercially available soybean varieties in terms of its safety for human consumption and nutritional adequacy;

- labelling of certain food fractions derived from glyphosate-tolerant soybean MON 89788 will be required if novel DNA and/or protein is present in the final food; and

- a regulation impact assessment process has been undertaken that also fulfils the requirement in New Zealand for an assessment of compliance costs. The assessment concluded that the most appropriate option is option 2, an amendment to the Code.

Consultation

The Initial Assessment was advertised for public comment between 13 December 2006 and 7 February 2007. A total of six submissions were received during this period. The Draft Assessment was advertised for public comment between 8 August 2007 and 19 September 2007. A total of nine submissions were received. A summary of these is provided in **Attachment 3** to this Report.

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INTRODUCTION

An Application was received from Monsanto Australia Limited on 19 October 2006 seeking approval for food derived from glyphosate-tolerant soybean (*Glycine max*) line MON 89788 under Standard 1.5.2 – Food produced using Gene Technology.

The genetic modification involved the transfer of the *cp4 epsps* gene into soybean. This gene is from a common soil bacterium and encodes the protein CP4-EPSPS (5-enolpyruvyl-3-shikimate phosphate synthase), which confers tolerance to the herbicide glyphosate.

A Final Assessment of the Application has been completed, including a comprehensive safety assessment and consideration of issues raised in public consultation.

1. Background

The genetic modification in glyphosate-tolerant soybean MON 89788 involves the introduction of the *cp4 epsps* gene derived from *Agrobacterium* sp. strain CP4. The *cp4 epsps* gene codes for an enzyme, 5-enolpyruvyl-3-shikimate phosphate synthase (EPSPS), which confers tolerance to the herbicide glyphosate. The EPSPS enzyme is present in all plants, bacteria and fungi and is essential for aromatic amino acid biosynthesis. The normal mode of action of glyphosate is to inhibit the endogenous plant EPSPS, thus blocking the synthesis of aromatic amino acids in cells which subsequently leads to the death of the plant. In contrast to the plant EPSPS, the bacterial EPSPS is able to function in the presence of glyphosate, therefore expression of CP4 EPSPS in the plant allows continued production of aromatic amino acids in the presence of the herbicide.

The purpose of the modification is to provide growers with an effective method for controlling weeds, together with enhanced yield potential relative to their previous herbicide tolerant product, soybean line 40-3-2. Food from soybean line 40-3-2 was approved in Australia and New Zealand in 2000. The Applicant states that soybean line MON 89788 has equivalent herbicide tolerance, and thus the same weed control benefits, as soybean 40-3-2. However, soybean line MON 89788 is reported to have a yield advantage due to improvements in transformation technology that have allowed the gene cassette to be directly transformed into an elite soybean line, thus accelerating further breeding improvements.

Glyphosate-tolerant soybean is not intended to be grown in Australia or New Zealand at this time and therefore food from MON 89788 will be present in imported foods only.

1.1 Current Standard

Standard 1.5.2 requires that genetically modified foods undergo a pre-market safety assessment before they may be sold in Australia and New Zealand. Foods that have been assessed under the Standard, if approved, are listed in the Table to clause 2 of the Standard.

1.2 Overseas approvals

Glyphosate-tolerant soybean MON 89788 has been approved for food and feed use and environmental release in the United States (US Food and Drug Administration and the USDA-Animal and Plant Health Inspection Service) and Canada (Health Canada and the Canadian Food Inspection Agency).

Regulatory submissions for import approvals have been or will be made to countries that import significant soybean or soybean products, including China, Japan, Korea, the Philippines and Taiwan.

2. The Issue / Problem

Before food derived from soybean line MON 89788 can enter the food supply in Australia and New Zealand, it must first be assessed for safety and an amendment to the Code must be approved by the FSANZ Board, and subsequently be notified to the Australia and New Zealand Food Regulation Ministerial Council (Ministerial Council). An amendment to the Code may only be gazetted, once the Ministerial Council process has been finalised.

Monsanto Australia Limited has therefore applied to have Standard 1.5.2 amended to include food derived from soybean line MON 89788.

3. Objectives

The objective of this assessment is to determine whether it would be appropriate to amend the Code to approve the use of food derived from soybean line MON 89788 under Standard 1.5.2. In developing or varying a food standard, FSANZ is required by its legislation to meet three primary objectives, which are set out in section 18 of the FSANZ Act. These are:

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

In developing and varying standards, FSANZ must also have regard to:

- the need for standards to be based on risk analysis using the best available scientific evidence;
- the promotion of consistency between domestic and international food standards;
- the desirability of an efficient and internationally competitive food industry;
- the promotion of fair trading in food; and
- any written policy guidelines formulated by the Ministerial Council.

4. Key Assessment Questions

Based on information provided by the Applicant on the nature of the genetic modification, the molecular characterisation, the characterisation of the novel protein, the compositional analysis and any nutritional issues, is food derived from soybean line MON 89788 as safe as that derived from conventional varieties of soybean?

Is there other available information, including from the scientific literature, general technical information, independent scientists, other regulatory agencies and international bodies, and the general community that needs to be considered?

Are there any other considerations that would influence the outcome of this assessment?

RISK ASSESSMENT

Food from soybean line MON 89788 has been evaluated according to the safety assessment guidelines prepared by FSANZ[1]. The summary and conclusions from the full safety assessment report (at **Attachment 2**) are presented below. In addition to information supplied by the Applicant, other available resource material including published scientific literature and general technical information was used for the assessment.

5. Risk Assessment Summary

In conducting a safety assessment of food derived from glyphosate-tolerant soybean MON 89788, a number of criteria were addressed including:

(i) characterisation of the transferred genes, their origin, function and stability;
(ii) changes at the level of DNA, protein and in the whole food;
(iii) compositional analyses, and an evaluation of intended and unintended changes; and
(iv) potential for the newly expressed proteins to be either allergenic or toxic in humans.

Detailed molecular and genetic analyses of glyphosate-tolerant soybean MON 89788 indicate that the transferred gene is stably integrated into the plant genome as a single copy at one insertion site, and is inherited in subsequent generations according to predicted patterns of inheritance. There was no transfer of bacterial antibiotic resistance marker genes in this modification.

The EPSPS protein present in glyphosate-tolerant soybean MON 89788 has been assessed previously for safety. These assessments have shown that CP4 EPSPS administered directly to animals at high doses is not toxic, and the evidence indicates no potential for this protein to be allergenic to humans. The novel EPSPS protein is expressed at moderate levels in glyphosate-tolerant MON 89788.

Compositional analyses of soybean grain did not reveal any meaningful differences between glyphosate-tolerant MON 89788 and its non-GM counterpart. The use of MON 89788 for food would be expected to have minimal nutritional impact.

Overall, no potential public health and safety concerns have been identified in the comprehensive assessment of glyphosate-tolerant soybean MON 89788. On the basis of the data provided in the present application, and other available information, food derived from glyphosate-tolerant soybean MON 89788 is considered as safe and wholesome as food derived from other soybean varieties.

[1] FSANZ (2003) Information for Applicants – Format for applying to amend the Australian New Zealand Food Standards Code – Food Produced using Gene Technology.

RISK MANAGEMENT

6. Options

FSANZ is required to consider the impact of various regulatory (and non-regulatory) options on all sectors of the community, which includes consumers, food industries and governments in Australia and New Zealand.

The two regulatory options available for this Application are:

6.1 Option 1 – *Status quo*

Maintain the *status quo* by not amending Standard 1.5.2 to approve the sale and use of food derived from glyphosate-tolerant soybean line MON 89788.

6.2 Option 2 – Approve food derived from soybean line MON 89788

Amend Standard 1.5.2 to permit the sale and use of food derived from glyphosate-tolerant soybean line MON 89788, with or without listing special conditions in the Table to clause 2 of Standard 1.5.2.

7. Impact Analysis

7.1 Affected Parties

The affected parties to this Application include the following:

- consumers, particularly those who have concerns about biotechnology;
- food importers and distributors of wholesale ingredients;
- the manufacturing and retail sectors of the food industry; and
- Government generally, where a regulatory decision may impact on trade or WTO obligations and enforcement agencies in particular who will need to ensure that any approved products are correctly labelled.

The cultivation of soybean line MON 89788 may have an impact on the environment, which would need to be assessed by the Office of the Gene Technology Regulator in Australia and by various New Zealand Government agencies including the Environmental Risk Management Authority and the Ministry of Agriculture and Forestry before cultivation in either of these countries could be permitted. At this stage, the Applicant has no plans for cultivation in either country.

7.2 Benefit Cost Analysis

In the course of developing food regulatory measures suitable for adoption in Australia and New Zealand, FSANZ is required to consider the impact of all options on all sectors of the community, including consumers, the food industry and governments in both countries.

The regulatory impact assessment identifies and evaluates, though is not limited to, the costs and benefits of the regulation, and its health, economic and social impacts.

Following public consultation on the Initial Assessment, FSANZ has identified the following potential costs and benefits of the two regulatory options:

7.2.1 Option 1 – status quo

Consumers: Cost in terms of a possible restriction in the availability of soybean products if MON 89788 soybean is present in imported foods.

No impact on consumers wishing to avoid GM foods, as food from glyphosate-tolerant soybean MON 89788 is not currently permitted in the food supply. However, food derived from glyphosate-tolerant soybean line 40-3-2 is permitted.

Government: No immediate impact.

Potential impact if considered inconsistent with WTO obligations but impact would be in terms of trade policy rather than in government revenue.

Industry: No immediate impact.

Cost in terms of restricting innovation in food/crop production for both growers and other sectors of the food industry. Cost to the food industry to source either segregated or non-GM supplies.

Possible restriction on soybean imports as MON 89788 soybean is already approved overseas and likely to be commercialised by 2009.

Potential longer-term impact - any successful WTO challenge has the potential to impact adversely on food industry.

7.2.2 Option 2 – approve food derived from glyphosate-tolerant soybean MON 89788

Consumers: No direct impact.

Possible benefit of lower prices, to the extent that savings from production efficiencies are passed on.

Benefit of access to a greater range of products including imported food products containing ingredients derived from soybean MON 89788.

Possible cost to consumers wishing to avoid GM food by a potential restriction of choice of products, or increased prices for non-GM food, although impact expected to be minimal as glyphosate-tolerant soybean line 40-3-2 is already widely cultivated.

Government: No direct impact. Benefit that if MON 89788 soybean was detected in soybean imports, approval would ensure compliance of those products with the Code.

This would ensure no potential for trade disruption on regulatory grounds.

Approval of MON 89788 soybean would ensure no conflict with WTO responsibilities.

This decision is likely to impact on monitoring resources of state, territory and New Zealand enforcement agencies, as certain foods derived from glyphosate-tolerant MON 89788 would be required to be labelled as genetically modified, increasing the costs incurred in monitoring for the presence of GM foods.

Industry: No direct impact. Approving soybean line MON 88972 will not have any significant cost implications and this is reflected by the Business Cost Calculator at **Attachment 4**.

Benefit to importers and distributors of overseas food products as the product range is extended.

Benefit for food manufacturers in that the choice of raw ingredients is extended.

Benefit to food retailers in an increased product range.

Benefit to importers of processed foods containing soybean as an ingredient as foods derived from MON 89788 soybean would be compliant with the Code.

Possible cost to food industry as some food ingredients derived from soybean MON 89788 will be required to be labelled as genetically modified.

7.3 Comparison of Options

As food from glyphosate-tolerant soybean MON 89788 has been found to be as safe as food from conventional varieties of soybean, option 1 is likely to be inconsistent with Australia and New Zealand's WTO obligations. Option 1 would also offer little benefit to consumers wishing to avoid GM foods, as approval of soybean MON 89788 by other countries could limit supplementation of the Australian and New Zealand market with imported soybean products. Option 1 is also unlikely to offer significant benefit to those consumers wishing to avoid GM foods as soybean MON 89788 is intended to supersede glyphosate-tolerant soybean 40-3-2, which is already widely cultivated and likely to be present in imported food products.

Under Option 2, primary producers would benefit from an increased choice of crop lines with potentially lower production costs and higher yields, which could flow on to other sectors including consumers in Australia and New Zealand as lower food prices. Given MON 89788 is already approved in the United States, option 2 would also have the benefit of minimising trade disruption in the event of co-mingling of MON 89788 with other approved varieties of GM soybean.

As MON 89788 soybean has been found to be safe for human consumption and the potential benefits outweigh the potential costs, Option 2, an amendment to Standard 1.5.2 of the Code giving approval to glyphosate-tolerant soybean MON 89788, is therefore the preferred option.

COMMUNICATION AND CONSULTATION STRATEGY

8. Communication

This is a routine approval matter. As a result, FSANZ has applied a basic communication strategy to Application A592. This involves advertising the availability of assessment reports for public comment in the national press and making the reports available on the FSANZ website. We will issue a media release drawing journalists' attention to the matter.

The Applicant and individuals and organisations who made submissions on this Application will be notified at each stage of the Application. Approval of the proposed variation to the Code will be notified to the Ministerial Council. The Applicant and stakeholders, including the public, will be notified of the gazettal of changes to the Code in the national press and on the website.

FSANZ provides an advisory service to the jurisdictions on changes to the Code.

9. Consultation

9.1 Public Consultation

The Initial Assessment was advertised for public comment between 13 December 2006 and 7 February 2007. Six submissions were received during this period. The Draft Assessment was advertised between 8 August 2007 and 19 September 2007. A total of nine submissions were received. A summary of these is provided in **Attachment 3** to this Report. FSANZ has taken the submitters' comments relevant to food safety into account in preparing the Final Assessment of this application. The main issues raised in public comments are discussed below.

9.1.1 Enforcement costs

The NSW Food Authority and Queensland Health have indicated that there are extensive costs incurred in monitoring for the presence of GM food, as detection of GM foods is more complex and expensive than other food regulatory measures. Both jurisdictions believe that the cost benefit analysis included in the DAR is insufficient and that there is a need to consider a national enforcement strategy surrounding GM food approvals. The NSW Food Authority have indicated that they intend to commence a process involving all jurisdictions to discuss this matter

9.1.1.1 Response

The costs associated with detecting GM from non-GM sources depends on the level of detail required for the investigation, as the number of introduced genetic traits is relatively small compared to the number of individually approved GM lines. Routine detection methods can distinguish a GM from a non-GM source when genetic material is present, however other analyses could be required for event-specific detection.

Costs associated with the enforcement by jurisdictions of any new food regulatory measure are considered by FSANZ in the Regulatory Impact Statement (RIS) and are not unique to GM foods.

Inevitably, enforcement costs would be expected to rise over time as a result of the need to regulate an ever-increasing number of new food additives, processing aids and novel technologies in the Code. Australia and New Zealand's current system of food regulation provides for the discussion of such issues by the Implementation Sub-Committee (ISC). FSANZ will work with the jurisdictions in developing a national enforcement strategy for GM food.

9.1.2 Possible presence of residual CTP2 targeting peptide

The Institute of Environmental Science and Research Limited (ESR) reviewed the Safety Assessment of A592 at the request of the New Zealand Food Safety Authority (NZFSA). As a result, NZFSA believes comment is required in the FAR on whether any assessment for residual CTP2 targeting peptide was performed, and if not a justification for the assumption that the peptide was fully degraded should be provided.

9.1.2.1 Response

The *cp4 epsps* coding sequence in soybean MON89788 is preceded by a chloroplast transit peptide sequence, *CTP2*, derived from the *Arabidopsis thaliana epsps* gene (Klee et al, 1987). The CTP2 transit peptide delivers CP4 EPSPS to the chloroplast and is subsequently cleaved from the pre–protein, yielding mature CP4 EPSPS with no CTP amino acids retained, as confirmed by biochemical analysis.

While it is generally accepted in the literature that chloroplast transit peptides are rapidly degraded after cleavage *in vivo* by cellular proteases, the section of the safety assessment dealing with characterisation of the novel protein has been amended to explain why it is unlikely that residual CTP2 peptide is present in the plant.

It is also worth noting that the CTP2 transit peptide from Arabidopsis has been used in a number of glyphosate tolerant GM crops, for example lucerne (A575), cotton (A355 and A553), corn (A416 and A548), sugar beet (A378 and A525) and canola (A363).

9.1.3 Inadequate labelling of foods derived from GM plants

Three private submissions (Ivan Jeray, Penelope Gordon and Paul Elwell-Sutton) stated that the labelling requirements for GM foods do not provide sufficient information to allow choice.

9.1.3.1 Response

Health Ministers on the former Australia New Zealand Food Standards Council (ANZFSC) resolved in July 2000 to require labelling of GM foods with the words 'genetically modified' where novel DNA and/or protein from an approved GM variety is present in the final food or where the food has altered characteristics. The Ministers resolved that highly refined food, such as oils, sugars and starches that have undergone refining processes that have the effect of removing DNA and/or protein, would be exempt from these requirements. The labelling provisions of Division 2 of Standard 1.5.2 (Appendix D) came into effect in December 2001.

All food produced using gene technology is required to undergo a pre-market safety assessment before sale and use in Australia and New Zealand. As the safety of GM food is assessed, labelling is primarily intended to provide information to facilitate consumer choice. GM food labelling allows consumers to purchase or avoid GM foods depending on their own views and beliefs. These general labelling requirements are based on the presence of novel DNA and/or protein in the food rather than on the process used.

9.2 World Trade Organization (WTO)

As members of the World Trade Organization (WTO), Australia and New Zealand are obligated to notify WTO member nations where proposed mandatory regulatory measures are inconsistent with any existing or imminent international standards and the proposed measure may have a significant effect on trade.

Guidelines for assessing the safety of GM foods have been developed by the Codex Alimentarius Commission and have the status of standards for WTO purposes. The proposed amendment to Standards 1.5.2 to allow food derived from soybean MON 89788 may be of interest to other WTO member nations because it pertains to the safety of GM food and is likely to have a liberalising effect on international trade.

For these reasons, notification was recommended to the agencies responsible in accordance with Australia's and New Zealand's obligations under the WTO Sanitary and Phytosanitary Measure (SPS) Agreements. Australia and New Zealand subsequently notified the WTO under the SPS Agreement to enable other WTO member countries to comment on the proposed changes to standards. No responses were received in response to the notification.

CONCLUSION

10. Conclusion and Decision

Decision

Amend Standard 1.5.2 - Food produced using Gene Technology, to include food derived from glyphosate-tolerant soybean MON 89788 in the Table to clause 2.

Reasons for Decision

An amendment to the Code approving food derived from glyphosate-tolerant soybean MON 89788 in Australia and New Zealand is approved on the basis of the available scientific evidence, for the following reasons:

- the safety assessment did not identify any public health and safety concerns associated with the genetic modification used to produce glyphosate-tolerant soybean MON 89788;

- food derived from glyphosate-tolerant soybean MON 89788 is equivalent to food from other commercially available soybean varieties in terms of its safety for human consumption and nutritional adequacy;

- labelling of certain food fractions derived from glyphosate-tolerant soybean MON 89788 will be required if novel DNA and/or protein is present in the final food; and

- a regulation impact assessment process has been undertaken that also fulfils the requirement in New Zealand for an assessment of compliance costs. The assessment concluded that the most appropriate option is option 2, an amendment to the Code.

11. Implementation and Review

It is proposed that the draft variation come into effect on the date of gazettal.

ATTACHMENTS

1. Draft variation to the *Australia New Zealand Food Standards Code*
2. Safety Assessment Report
3. Summary of issues raised in public submissions
4. Business Cost Calculator Report

Attachment 1

Draft variation to the *Australia New Zealand Food Standards Code*

Standards or variations to standards are considered to be legislative instruments for the purposes of the Legislative Instruments Act (2003) and are not subject to disallowance or sunsetting.

To commence: on gazettal

[1] ***Standard 1.5.2*** *of the Australia New Zealand Food Standards Code is varied by inserting in the* Table to clause 2 –

Food derived from glyphosate-tolerant soybean line MON 89788	

Attachment 2

SAFETY ASSESSMENT

APPLICATION A592: FOOD DERIVED FROM GLYPHOSATE-TOLERANT SOYBEAN MON 89788

SUMMARY AND CONCLUSIONS

Background

Glyphosate-tolerant soybean MON 89788 has been genetically modified for tolerance to the broad-spectrum herbicide glyphosate. Tolerance is conferred by expression of the *cp4 epsps* gene in the soybean crop.

An earlier version of glyphosate-tolerant soybean, line 40-3-2, is already approved under Standard 1.5.2. Soybean line 40-3-2 currently accounts for 60% of the global production of soybean. Glyphosate-tolerant soybean MON 89788 is claimed to provide enhanced yield potential relative to soybean line 40-3-2. Glyphosate-tolerant soybean MON 89788 was developed primarily for cultivation in the United States and is not intended for cultivation in Australia or New Zealand. Australia and New Zealand import a considerable quantity of soybean and soybean products from the United States. Therefore, it is likely that, if approved, imports of soybean and soybean products into Australia and New Zealand will contain MON 89788.

In conducting a safety assessment of food derived from glyphosate-tolerant soybean MON 89788, a number of criteria have been addressed including: a characterisation of the transferred genes, their origin, function and stability in the soybean genome; the changes at the level of DNA, protein and in the whole food; compositional analyses; evaluation of intended and unintended changes; and the potential for the newly expressed proteins to be either allergenic or toxic in humans.

This safety assessment report addresses only food safety and nutritional issues. It therefore does not address: environmental risks related to the environmental release of GM plants used in food production; the safety of animal feed or animals fed with feed derived from GM plants; or the safety of food derived from the non-GM (conventional) plant.

History of Use

The cultivated soybean, *Glycine max* (L.) Merr., is an annual crop grown commercially in over 35 countries. Soybean is the dominant oilseed traded in international markets (OECD, 2001). There are three major soybean products — beans, meal and oil. The primary use of soybean meal is in animal feed, although a proportion is also used for human food products. The principle processed fraction used by the food industry is soybean oil. There are no human food uses for raw unprocessed soybeans as they contain high levels of trypsin inhibitor which has anti-nutritional properties. A significant proportion of the trypsin inhibitor is destroyed by heat treatment.

Description of the Genetic Modification

Glyphosate-tolerant soybean MON 89788 was generated through the transfer of the *cp4 epsps* gene to the elite soybean line, A3244. Direct transfer into elite germplasm accelerates subsequent introgression of the trait into other soybean varieties and is reported to provide a yield advantage compared to the already approved glyphosate-tolerant soybean 40-3-2.

The *cp4 epsps* gene is derived from the soil bacterium *Agrobacterium sp*. strain CP4 which encodes a version of the enzyme 5-enolpyruvyl-3-shikimatephosphate synthase (CP4 EPSPS). Unlike the plant's own EPSPS, CP4 EPSPS continues to function in the biochemical pathway producing aromatic amino acids in a plant that has been treated with glyphosate. There was no transfer of bacterial antibiotic resistance marker genes in this modification.

Detailed molecular and genetic analyses of glyphosate-tolerant soybean MON 89788 indicate that the transferred gene is stably integrated into the plant genome as a single copy and is inherited in subsequent generations according to predicted patterns of inheritance.

Characterisation of Novel Protein

The mature CP4 EPSPS in glyphosate-tolerant soybean MON 89788 is identical to the bacterial enzyme of 455 amino acids and is targeted to the plant chloroplast, the site of synthesis of essential aromatic compounds.

The novel protein is expressed at moderate levels in glyphosate-tolerant MON 89788 soybean plants. The mean level of CP4 EPSPS in grain (seed) was 140 μg/g fresh weight and 150 μg/g dry weight. This is lower than the level of CP4 EPSPS protein in the previous glyphosate-tolerant soybean 40-3-2 (average 288 μg/g fresh weight).

Potential toxicity and allergenicity

The novel protein present in glyphosate-tolerant soybean MON 89788 has been assessed previously for safety; the CP4 EPSPS protein is present in approved lines of canola, cotton, soybean, potato, corn and lucerne. Previous assessments have shown that CP4 EPSPS administered directly to animals at a high dose is not toxic, and the evidence does not indicate any potential for this protein to be allergenic in humans. Given its widespread use in approved glyphosate-tolerant crops, CP4 EPSPS now has a history of safe use in food over 10 years.

Compositional Analyses

Compositional studies were conducted to establish the nutritional adequacy of glyphosate-tolerant soybean MON 89788 compared to the non-GM control and conventionally produced commercial soybean varieties. Components measured in grain samples were proximates (protein, fat, ash and moisture), acid detergent fibre (ADF), neutral detergent fibre (NDF), amino acids, fatty acids (C8-C22), phytic acid, trypsin inhibitor, isoflavones, lectins, farinose, stachyose, vitamin E and carbohydrates (by calculation).

In general, no differences of biological significance were observed between glyphosate-tolerant soybean MON 89788 and its non-GM counterpart. Food from glyphosate-tolerant soybean MON 89788 is therefore considered to be compositionally equivalent to food from the control and commercially available soybean varieties.

Soybean is known to be one of the major allergenic foods. The potential allergenicity of soybean MON 89788 was compared to that of commercially available soybean varieties by assessing IgE binding responses using sera from known soybean allergic patients. These studies indicated that soybean MON 89788 does not have any greater potential to be allergenic than commercially available soybean varieties.

Nutritional Impact

The detailed compositional studies are considered adequate to establish the nutritional adequacy of food derived from glyphosate-tolerant soybean MON 89788. The introduction of glyphosate-tolerant soybean MON 89788 into the food supply would be expected to have minimal nutritional impact. This was supported by the results of a broiler feeding study, where no difference was found between birds fed diets containing MON 89788 soybean meal and those birds fed conventional soybean meal diets.

Conclusion

No potential public health and safety concerns have been identified in the comprehensive assessment of glyphosate-tolerant soybean MON 89788. On the basis of the data provided in the present application, and other available information, food derived from glyphosate-tolerant soybean MON 89788 is considered as safe and wholesome as food derived from other soybean varieties.

1. INTRODUCTION

Monsanto Australia Ltd is seeking approval in Australia and New Zealand for food derived from a genetically modified herbicide-tolerant soybean MON 89788 under Standard 1.5.2 – Food produced using Gene Technology in the *Australia New Zealand Food Standards Code*. Soybean MON 89788 has been modified for tolerance to the broad-spectrum herbicide glyphosate. The intended product name for this soybean is Roundup RReady2Yield™.

Soybean (*Glycine max* (L.) Merr) is an annual crop grown for meal and oil. The primary use of soybean meal is in animal feed, although a proportion can also be used for human food products. The principle processed fraction used by the food industry is soybean oil. There are no human food uses for unprocessed soybeans as they contain high levels of trypsin inhibitor which has anti-nutritional properties. A significant proportion of the trypsin inhibitor is destroyed by heat treatment.

The glyphosate tolerance trait in soybean MON 89788 is due to the expression of the bacterial enzyme 5-enolpyruvyl-3-shikimate phosphate synthase (EPSPS) from *Agrobacterium sp*. strain CP4. The EPSPS enzyme is present in all plants, bacteria and fungi and is essential for aromatic amino acid biosynthesis. The normal mode of action of glyphosate is to bind to the endogenous plant EPSPS, blocking its enzymatic activity which subsequently leads to the death of the plant.

The bacterial EPSPS enzyme has a lower binding affinity for glyphosate, and therefore expression of CP4 EPSPS in the plant allows continued production of aromatic amino acids in the presence of the herbicide.

Glyphosate-tolerant soybean enables the use of herbicides to provide effective weed control during forage and seed production. An existing glyphosate-tolerant soybean, 40-3-2, currently accounts for 60% of the global soybean area and is the most cultivated genetically modified plant product to date. This new version of glyphosate-tolerant soybean exploits improvements in biotechnology and molecular-assisted breeding to enhance yield by 4-7% compared to the existing variety, while maintaining effective weed control. This was achieved by directly transforming the glyphosate-tolerant trait into an elite soybean variety with favourable agronomic characteristics and high yields, allowing more efficient introgression of the trait into other soybean varieties.

2. HISTORY OF USE

2.1 Donor organisms

Agrobacterium sp. strain CP4 produces a naturally glyphosate-tolerant EPSPS enzyme and was therefore chosen as a suitable gene donor for the herbicide tolerance trait (Padgette *et al*., 1996). The bacterial isolate CP4 was identified in the American Type Culture Collection as an *Agrobacterium* species. *Agrobacterium* species are known soil-borne plant pathogens but are not pathogenic to humans or other animals.

2.2 Host organism

Cultivated soybean (*Glycine max* (L.) Merr) is a diploidised tetraploid (2n=40) of the Leguminosae family. Soybean is an annual crop that is grown commercially in over 35 countries world-wide. Soybean is the major oilseed crop in terms of world production and trade in international markets. In 2005-2006 global production exceeded 219 million metric tons. The major producers are the US, Argentina, Brazil and China; these countries account for 87% of total production (OECD, 2001). In 2005, glyphosate-tolerant soybean line 40-3-2 accounted for 60% of global soybean production (James, 2005).

The majority of soybean is processed for soybean meal used in animal feed, and soybean oil for human food uses. Soybeans are a traditional source of protein and oil for human consumption. Foods that contain soybean protein include bakery products, confections, meat products, textured foods and nutritional supplements. Soybean protein isolate is also the protein source for soy–based infant formula, where the amino acid and fatty acid profile is very important (OECD, 2001). The oil is typically used in margarine, shortening, cooking oil, salad oil and mayonnaise. Lecithin, derived from crude soybean oil, is used as a natural emulsifier, lubricant and stabilising agent.

There are no human food uses for raw unprocessed soybeans as they contain high levels of trypsin inhibitor and lectins, both of which have anti-nutritional properties. A significant proportion of both trypsin inhibitor and lectins is destroyed by heat treatment. Phytic acid present in soybean can reduce bioavailability of some mineral nutrients (OECD, 2001).

Soybean also contains phytoestrogens, naturally occurring isoflavone compounds that have a number of biochemical activities in mammals. The low molecular weight carbohydrates stachyose and raffinose are the cause of gas production resulting in flatulence and are considered to be anti-nutrients.

Soybeans contain several allergenic proteins that can cause severe adverse reaction when present in the diet of hypersensitive individuals (OECD, 2001).

3. DESCRIPTION OF THE GENETIC MODIFICATION

3.1 Method used in the genetic modification

MON 89788 was generated by *Agrobacterium*-mediated transformation of meristem tissue of Asgrow soybean variety A3244, based on the method developed by Martinell et al. (Martinell *et al.*, 2002).

The *Agrobacterium*-mediated DNA transformation system is the basis of natural plasmid-induced crown-gall formation in many plants and is well understood (Zambryski, 1992). The genes of interest were inserted into the plasmid between DNA sequences known as the Left and Right Borders (LB and RB). These border sequences were isolated from the Ti plasmid of *Agrobacterium* and normally delimit the DNA sequence (T-DNA) transferred into the plant.

A double border, binary vector PV-GMGOX20, was used to generate transformation event MON 89788. This vector contains the *cp4 epsps* coding region under the control of a constitutive promoter. PV-GMGOX20 also contains both the left and right transfer-DNA (T-DNA) border sequences to facilitate transformation. The genetic elements present in PV-GMGOX20 are shown in Table 1. *Agrobacterium tumefaciens* strain ABI was used as it contains a disarmed Ti plasmid that is incapable of inducing tumour formation because of the deletion of the phytohormone genes originally present in the *Agrobacterium* Ti plasmid.

Table 1: Genetic elements in plasmid PV- GMGOX20

Genetic element	Size in base pairs (position in plasmid)	Function
Intervening sequence	51 (1-51)	Sequences used in DNA cloning
FMV/Tsf1	1040 (52-1091)	Chimeric promoter consisting of the enhance sequences from the 35S promoter of the Figwort Mosaic virus (Richins *et al*., 1987) and the promoter from *Tsf1* of *Arabidopsis thaliana* encoding elongation factor EF-1 alpha (Axelos *et al*., 1989)
Tsf1	46 (1092-1137)	5' non-translated leader (exon 1) from *Tsf1* of *Arabidopsis thaliana* encoding elongation factor EF-1 alpha (Axelos *et al*., 1989)
Tsf1	622 (1138-1759)	Intron from *Tsf1* of *Arabidopsis thaliana* encoding elongation factor EF-1 alpha (Axelos *et al*., 1989)
Intervening sequence	9 (1760-1768)	Sequences used in DNA cloning
CTP2	228 (1769-1996)	Chloroplast transit peptide sequence from the ShkG gene of *A. thaliana* (Klee *et al*., 1987)

Genetic element	Size in base pairs (position in plasmid)	Function
cp4 epsps	1368 (1997-3364)	Codon optimised coding sequence of the aroA (epsps) gene from Agrobacterium sp. Strain CP4 (Padgette *et al*., 1996; Barry *et al.*, 1997)
Intervening Sequence	42 (3365-3406)	Sequences used in DNA cloning
E9	643 (3407-4049)	3' untranslated sequence from the ribulose-1,5-bisphosphate carboylase small subunit (*RbcS2*) *E9* gene from pea (*Pisum sativum*). Transcriptional termination sequence and polyadenylation signal sequence (Coruzzi *et al*., 1984)
Intervening sequence	43 (4050-4092)	Sequences used in cloning
Left Border	442 (4093-4534)	Left border sequence essential for T-DNA transfer (Barker *et al*., 1983)
Intervening sequence	86 (4535-4620)	Sequences used in cloning
ori-V	397 (4621-5017)	Origin of replication for maintenance of the plasmid in Agrobacterium (Stalker *et al*., 1981)
Intervening sequence	1508 (5018-6525)	Sequences used in cloning
rop	192 (6526-6717)	Coding sequence for repressor of primer protein for maintenance of plasmid copy number in *E. coli* (Giza and Huang, 1989)
Intervening sequence	417 (6718-7134)	Sequences used in cloning
ori-PBR322	629 (7135-7763)	Origin of replication from pBR322 for maintenance of plasmid in *E. coli* (Sutcliffe, 1978)
Intervening sequence	500 (7764-8263)	Sequences used in cloning
aadA	889 (8264-9152)	Bacterial promoter and coding sequence for an aminoglycoside modifying enzyme, 3'(9)-O-nucleotidyltransferase from the transposon Tn7 (Fling *et al*., 1985)
Intervening sequence	136 (9153-9288)	Sequences used in cloning
Right Border	357 (9289-9645)	Right border sequence essential for T-DNA transfer (Depicker *et al*., 1982)
Intervening sequence	19 (9646-9664)	Sequences used in cloning

Following transformation, the meristems were placed on selection media containing glyphosate to inhibit the growth of untransformed plant cells. Carbenicillin and Claforan were used to prevent the growth of remaining Agrobacterium. The meristems were then placed in media conducive to root and shoot formation, and only those plants with normal phenotypic characteristics were selected and transferred to soil for growth and further assessment.

R0 plants were self-pollinated and the subsequent R1 plants screened for the presence of the CP4-EPSPS protein, tolerance to glyphosate and for the homozygosity of the inserted gene. The progeny of the glyphosate-tolerant, homozygous plants were subjected to further molecular and phenotypic analysis. Based on its superior phenotypic characteristics and molecular profile, MON 89788 was selected for further characterisation.

These steps are summarised in Figure 1. The breeding tree of MON 89788 is shown in Figure 2.

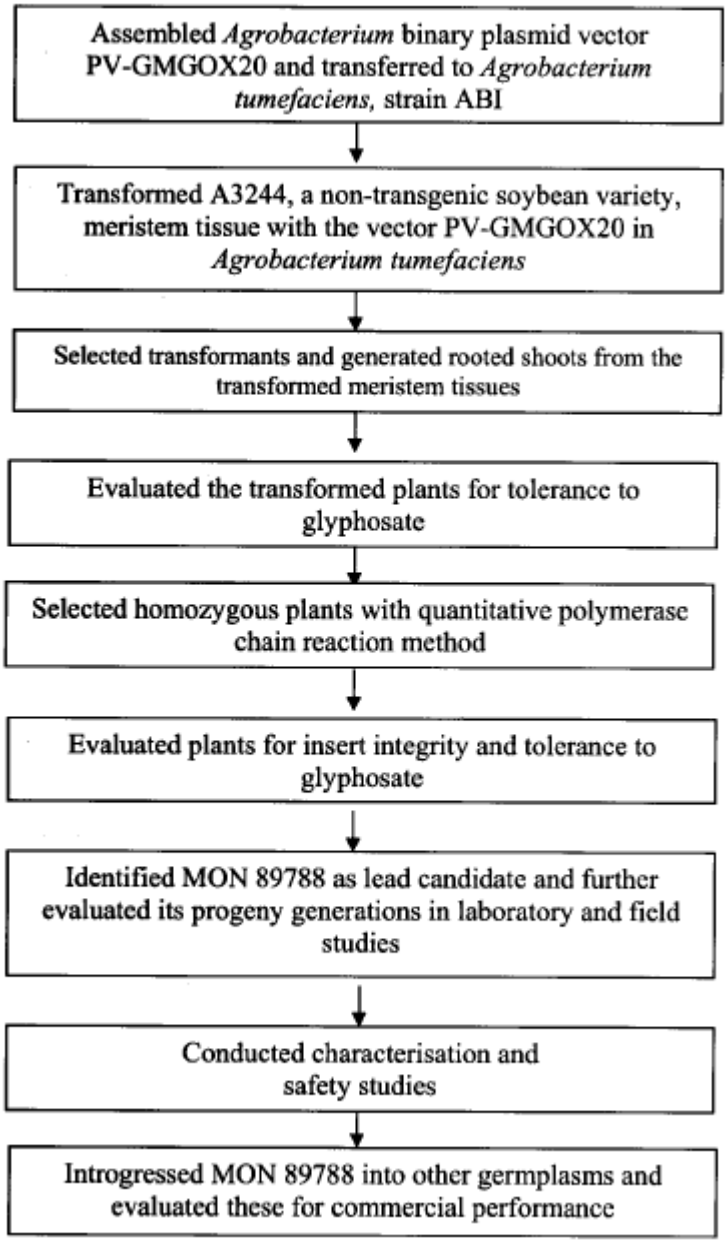

Figure 1: Development of MON 89788

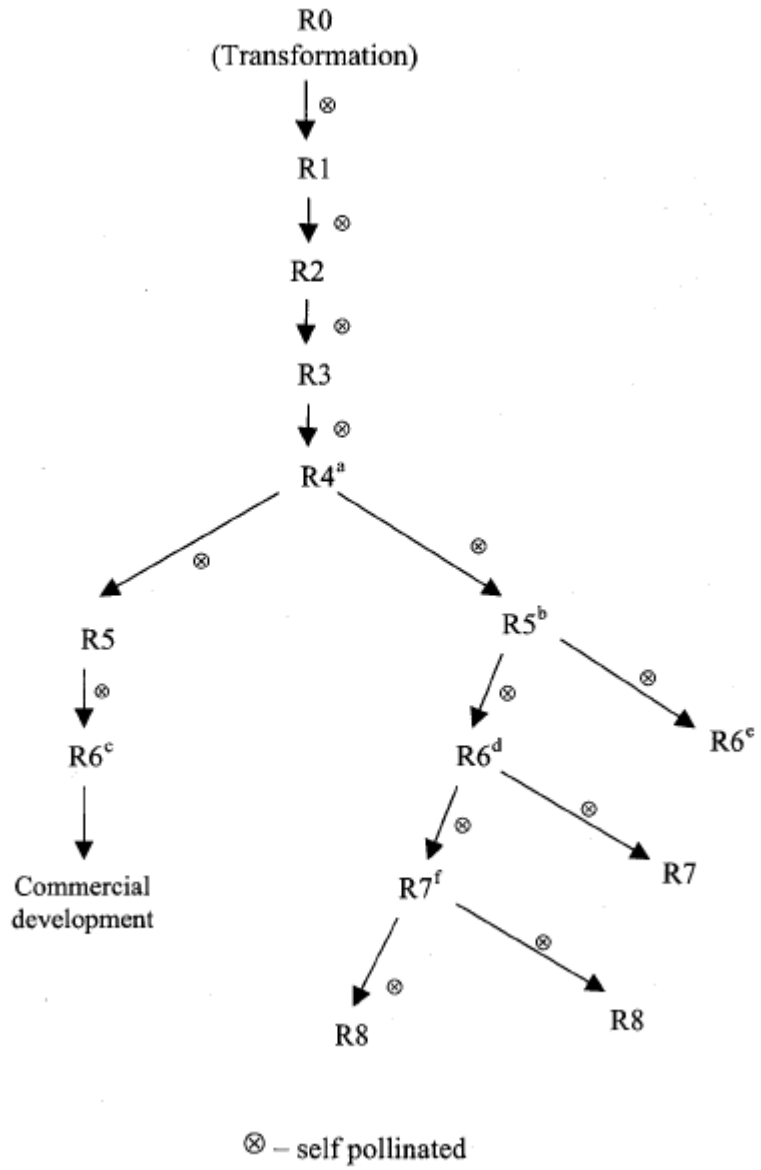

Figure 2: Breeding tree of MON 89788

Molecular characterisation was performed using $R5^b$ generation. Generational stability analyses were performed on $R4^a$, $R5^b$, $R6^c$, $R6^d$, $R6^e$ and $R7^f$.

3.2 Genetic elements in vector

Plasmid PV-GMGOX20 is approximately 9.7 kb and contains a *cps-epsps* gene expression cassette within the left and right border regions. Approximately 5.4 kb of this vector is backbone DNA and is not intended for incorporation into the soybean genome.

The *cp4-epsps* expression cassette T-DNA contains a chimeric transcriptional promoter (P-*FMV/Tsf1*), and leader and an intron sequence derived from the *Tsf1* gene (L-*Tsf1* and I-*Tsf1*), a chloroplast transit peptide sequence (TS-*CTP2*), the *cp4 epsps* coding sequence (CS-*cp4 epsps*), and a polyadenylation sequence from the RbcS2 gene (T-*E9*). This expression cassette is identical to that used in the transformation of Roundup Ready Flex cotton MON 88913, which was approved by FSANZ in 2006 (Application A553).

All genetic elements are shown in Table 1.

3.3 Function and regulation of the novel genes

The only novel gene introduced into soybean MON 89788 is *cp4 epsps*. Expression of the *cp4 epsps* gene in the soybean plants confers tolerance to the herbicide glyphosate.

Since the early 1990s it has been known that the *cp4 epsps* gene from *Agrobacterium* sp. strain CP4 has the potential to provide high levels of tolerance to glyphosate when introduced into plants. Glyphosate normally binds to the plant EPSPS enzyme, blocking biosynthesis of essential aromatic amino acids by the shikimate pathway, which is common to plants, bacteria and fungi. The bacterial CP4 EPSPS protein has a lower binding affinity with glyphosate compared to most other EPSPS enzymes and therefore retains its high catalytic efficiency in the presence of the herbicide. The bacterial *cp4 epsps* gene has been modified to optimise codon usage, which allows for higher expression in plants. These changes to the DNA sequence produce an identical CP4 EPSPS protein (Harrison *et al*., 1996) and do not affect the functional activity of the expressed protein.

Expression of *cp4-epsps* is regulated by the chimeric promoter *FMV/Tsf1*, which directs constitutive expression of *cp4 epsps* in soybean, conferring tolerance to the herbicide at the whole plant level.

The activity of the EPSPS enzyme in higher plants occurs in the chloroplast (la-Cioppa *et al*., 1986). The CP4 EPSPS protein is produced in the cytoplasm and then targeted to the chloroplasts via an N-terminal fusion with a chloroplast transit peptide sequence (CTP2). The CTP is typically cleaved on uptake of the mature protein into the chloroplast, and is subsequently rapidly degraded.

The *cp4 epsps* gene together with these plant regulatory elements has been used previously to confer glyphosate-tolerance in a range of food crops including canola, cotton, soybean, sugarbeet, and corn.

3.4 Characterisation of the novel gene in soybean MON 89788

Studies submitted:
Dickinson, E.C., N.G. Pineda, N.K. Scanlon, A.J. Whetsell and J.D. Masucci (2006) Molecular Analysis of Glyphosate-Tolerant Soybean MON 89788. Unpublished Monsanto Report MSL-20160

Masucci, J.D. (2006) Alignment of the MON 89788 Insert DNA Sequence to the PV-GMGOX20 Transformation Vector DNA Sequence. Unpublished Monsanto Report 06-RA-30-01

Insert and copy number

Analysis of the DNA introduced into glyphosate-tolerant soybean MON 89788 was undertaken using a range of established molecular techniques. Southern blot analyses were performed on genomic DNA extracted from soybean MON 89788 and the parent soybean cultivar A3244 as a control to assess the following:

(i) number of insertions of the expression cassette;
(ii) number of copies of the expression cassette;
(iii) integrity of the inserted gene expression cassette;

(iv) presence or absence of plasmid backbone; and
(v) stability of the inserted DNA with conventional breeding over several generations.

Genomic DNA from soybean MON 89788 and A3244 was digested with a variety of restriction endonucleases and subjected to Southern blot analyses. The plasmid PV-GMGOX20 was used as a reference substance serving as a positive hybridisation control. The Southern blot hybridisations, based on the method described by Southern (Southern, 1975), involved both short and long gel runs in order to improve the resolution of different size molecular fragments. Individual Southern blots were tested with probes corresponding to *cp4 epsps*, the promoter and polyadenylation sequence, and the transforming plasmid backbone. In all, ten radiolabelled probes corresponding to segments of DNA spanning the entire length of the plasmid PV-GMGOX20 were used in the analyses.

The combined results from these multiple Southern blot analyses indicate that glyphosate-tolerant soybean MON 89788 is characterised by the presence of one copy of the gene cassette, inserted at a single locus in the soybean genome. No unexpected hybridisation bands were detected. These results suggest that soybean MON 89788 does not contain any additional DNA elements other than those expected from the insertion of the *cp4 epsps* expression cassette. Fragments corresponding to partial genes, regulatory elements or backbone sequences derived from the transforming plasmid were not detected. A map of the inserted DNA presented below (Figure 3).

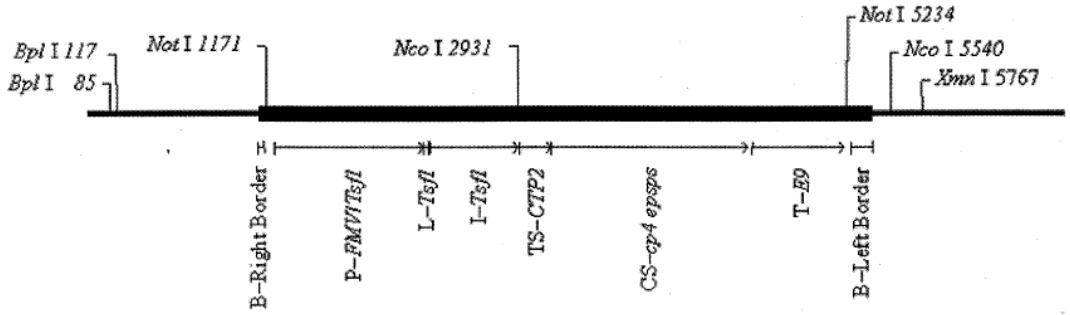

Figure 3: Map of the insertion event in glyphosate-tolerant soybean MON 89788
The bold heavy line represents the genetic material inserted into the soybean genome. The lighter line to the left and right of the insert represents genomic DNA. Individual genetic elements are identified below the insert. The map was developed on the basis of Southern blot characterisation data and confirmed by DNA sequence analysis.

PCR and sequence analysis

The organisation of the elements within the MON 89788 insert was confirmed by PCR analysis of three overlapping regions of DNA that span the entire length of the insert and soybean genomic flanking regions. Sequence analysis demonstrated that the sequence of the insert (4303 base pairs) is identical to that of the gene construct in the transforming plasmid. The insert begins at base 9604 of plasmid PV-GMGOX20, located in the right border region, and ends at base 4242 in the left border region. This sequence analysis confirmed the presence and organisation of the insert as shown in Figure 3.

Flanking regions and putative Open Reading Frame (ORF) analysis

Studies submitted:
Dickinson and Masucci (2006) PCR and DNA Sequence Analysis of Conventional Soybean to Examine the MON 89788 Insertion Site. Unpublished Monsanto Technical Report MSL-20320.

Soybean genomic DNA on either side of the MON 89788 *cp4 epsps* insert was also sequenced. Such alignment can reveal potential deletion or addition of DNA sequence in comparison to the wild-type genome at the site of the insertion event. PCR amplification of soybean A3244 genomic DNA using primers that flank the *cp4 epsps* insertion site of MON 89788 yielded a DNA product of ~650 base pairs which was sequenced using the same primers used for amplification. The A3244 sequence was compared to 1103 base pairs of MON 89788 genomic DNA at the 5' end of the transgene insert and 1060 base pairs at the 3' end of the insert. Results from the DNA sequence comparison indicated that 40 base pairs of parental genomic DNA were deleted from the site of the T-DNA insertion. In addition, there are ten bases at the 5' end and six bases at the 3' end of the insert that are not present in this region of the parental soybean genome. This minor deletion and insertion of DNA can occur due to double strand break repair mechanisms in the plant during the *Agrobacterium*-mediated transformation process (Salomon and Puchta, 1998). Based on this analysis, it can be concluded that there is minor rearrangement of the endogenous soybean genomic DNA at the MON 89788 insertion site, and that the DNA sequences flanking the insert are native to the soybean genome. The junction regions between the insert and genomic DNA were further analysed for their potential to be involved in the production of chimeric proteins.

The production of unexpected chimeric proteins as a result of transgene insertion is of particular relevance to food safety. In cases where there is 100% molecular identity between the plasmid T-DNA and inserted DNA in the plant, and all regulatory elements including termination and polyadenylation signals are intact, there is little likelihood of unintended formation of gene fragments that are transcriptionally active or likely to produce a chimeric protein.

In the case of glyphosate-tolerant soybean MON89788, the transformation event has not resulted in any additions, deletions, rearrangements or partial insertions of the gene of interest, or its regulatory elements, as determined by the Southern blot, PCR analyses and direct DNA sequencing of the entire insert region. Nonetheless, the applicant has provided a bioinformatic evaluation of DNA sequences flanking the junctions of the inserted DNA in MON 89788 for assessment of putative polypeptides. Two of the novel open reading frames between stop codons were less than eight amino acids, so bioinformatics analysis of the other ten putative open reading frames was performed using the ALLPEPTIDES, TOXIN5 and AD6 (the allergen database) databases. This is discussed further in section 4.6.

3.5 Stability of the genetic changes

Segregation data

During the development of MON 89788, R0 plants were self-pollinated and the resulting R1 plants segregated with the expected 3:1 ratio based on the glyphosate tolerance phenotype. Selected R1 plants that survived glyphosate treatment were subjected to quantitative PCR analysis and a single plant that was homozygous for the *cp4 epsps* expression cassette was selected.

Self-pollination of this plant gave rise to the R2 generation, with an expected segregation ratio for this and subsequent self pollinated generations of 100% positive for glyphosate tolerance. Phenotypic frequency was compared by Chi square analysis, which confirmed that the inserted *cp4 epsps* cassette was segregating as expected (Table 2). These results are consistent with a single insertion event segregating according to Mendel's laws of genetics.

Table 2: Genotypic Segregation Data for MON 89788

Generation	No. of Plant (% germ)[1]	Expected[2]		Observed[3]		χ^2
		Positive	Negative	Positive	Negative	
R1	43	32.25	10.75	29	14	1.31[4]
R2	58	58	0	58	0	Fixed
R3	240 (80%)	192	0	192[5]	0	Fixed
R3	240 (85%)	204	0	204[5]	0	Fixed
R3	240 (85%)	204	0	204[5]	0	Fixed

[1] Percent germination
[2] Expected number of glyphosate tolerant plants
[3] Observed number of glyphosate tolerant plants by ELISA and glyphosate application
[4] Not significant at $p \leq 0.05$ (Chi-square = 3.84 at 1 df)
[5] Number of plants (observed positives) was calculated based on number of seeds planted x percent germination

Stability of the inserted DNA

In order to demonstrate the stability of the genetic change in MON 89788 over multiple generations, additional Southern blot analyses were performed. Genomic DNA from four generations of MON 89788 was examined (R4 – R7, see Figure 2). Genomic DNA from the parental line A3244 and plasmid PV-GMGOX20 were used as negative and positive controls respectively.

Probes were used that spanned the insert region and the plasmid backbone region. The hybridisation patterns indicated that the insert in MON 89788 is stably integrated into the soybean genome. No plasmid backbone DNA was detected in any of the four generations assessed.

3.6 Antibiotic resistance genes

No antibiotic marker genes are present in glyphosate-tolerant soybean MON89788. The molecular characterisation shows that the region outside the T-DNA of plasmid PV-GMGOX20 was not integrated into the soybean genome during transformation. Consequently, the bacterial selectable marker gene, *aad* (which confers resistance to the antibiotics spectinomycin and streptomycin), is not present in glyphosate-tolerant soybean MON 89788. The absence of the bacterial marker gene in the plant was confirmed by Southern hybridisation analysis using a probe encompassing the *aad* gene.

4. CHARACTERISATION OF NOVEL PROTEINS

4.1 Function and phenotypic effects

Expression of the CP4 EPSPS protein in MON 89788 plants confers tolerance to the herbicide glyphosate. This protein is one of many EPSPS proteins found in nature in a broad range of organisms including plants, bacteria and fungi. The bacterial CP4 EPSPS is naturally highly tolerant to inhibition by glyphosate and continues to have high catalytic efficiency in the presence of the herbicide. Plant cells producing the CP4 EPSPS protein are therefore tolerant to glyphosate because the enzyme continues to function when the plant's own EPSPS has been inactivated by the herbicide.

Several glyphosate-tolerant varieties of corn, canola and soybean expressing CP4 EPSPS have been assessed for safety previously and are permitted on the market for use in food.

The mature 47.6 kDa CP4 EPSPS protein consists of a single polypeptide of 455 amino acids.

4.2 Protein Expression Analysis

Study submitted:
Pineda N.G. and A. Silvanovich (2006) Assessment of CP4 EPSPS Protein Quantities in Leaf, Seed, Root, and Forage Tissues from Second Generation Glyphosate-Tolerant Soybean MON 89788 Produced in the U.S. During 2005. Unpublished Monsanto study report MSL-20182

The levels of the CP4 EPSPS protein in leaf, seed, root and forage tissue of soybean MON 89788 were estimated using an enzyme-linked immunosorbent assay (ELISA). For capture of CP4 EPSPS, mouse monoclonal antibodies were used. A goat polyclonal CP4 EPSPS antibody was used for detection, with quantitation of protein levels accomplished by interpolation from a CP4 EPSPS protein standard curve. The limit of detection of the ELISA was estimated to be 0.26 µg/g fresh weight.

To produce the material for analysis, MON 89788 was planted at five field sites in the 2005 growing season. Sites represented geographies where soybean is typically grown in the United States – York County (Nebraska), Clinton County (Illinois), Warren County (Illinois), Jackson County (Arkansas), and Fayette County (Ohio).

Leaf tissue was collected four times over the growing period: OSL1 (over-season leaves V3-V4 growth stage); OSL2 (V6-V8 growth stage); OSL3 (V10-V12); an OSL4 (V14-V16 growth stage). Grain, root and forage were collected at one time point only. 15 samples were analysed for each tissue, except for forage, where only 14 samples were analysed. The mean levels of the CP4 EPSPS protein across the sites for OSL1, OSL2, OSL2, OSL4, grain, root and forage were 300, 340, 330, 290, 150, 74 and 220 µg/g dry weight respectively (Table 3).

The levels of CP4 EPSPS from the control soybean (conventional parental variety A3244) were less than the assay limit of detection in all tissue types.

Table 3: Summary of CP4 EPSPS protein levels in tissues collected from MON 89788 produced in the US during 2005

Tissue Type	CP4 EPSPS µg/g FW (SD)[1]	Range[2] (µg/g FW)	CP4 EPSPS µg/g DW (SD)[3]	Range (µg/g DW)	LOQ / LOD (µg/g FW)
OSL1[4]	54 (7.8)	40 – 66	300 (51)	220 – 380	0.57 / 0.26
OSL2[4]	60 (10)	42 – 80	340 (55)	250 – 440	0.57 / 0.26
OSL3[4]	58 (11)	40 – 79	330 (94)	200 – 520	0.57 / 0.26
OSL4[4]	75 (17)	60 – 110	290 (48)	210 – 390	0.57 / 0.26
Grain	140 (20)	98 – 170	150 (22)	110 – 180	0.34 / 0.26
Root	22 (6.0)	13 – 38	74 (27)	41 – 150	0.57 / 0.11
Forage	59 (14)	41 – 94	220 (51)	140 – 330	0.57 / 0.10

1. Protein quantities are expressed as mean µg of CP4 EPSPS/g tissue on a fresh weight (FW) basis. The mean and standard deviation (SD) were calculated across all sites.
2. Minimum and maximum values across all sites.
3. Protein quantities are expressed as mean µg of CP4 EPSPS/g tissue on a dry weight (DW) basis. The dry weight values were calculated by dividing the fresh weight values by the dry weight conversion factors obtained from moisture analysis data.
4. OSL1 to OSL4 represent over-season leaves collected at the following developmental stages: OSL1: V3-V4 growth stage; OSL2: V6-V8 growth stage; OSL3: V10-V12 growth stage; OSL4: V14-V16 growth stage.

Note: Sample number is 14 for forage, and 15 each for OSL1 to OSL4, grain, and root.

4.3 Characterisation of the novel protein in MON 89788

Studies submitted:
Kurunanandaa K, B.E. Goertz, J.J. Thorp, S.H. Elliot, and M. Alibhai (2006) Characterization of the CP4 EPSPS Protein Purified from the Seed of MON 89788 and Assessment of the Physicochemical and Functional Equivalence of the Plant and *E. coli*- Produced CP4 EPSPS Proteins. Unpublished Monsanto Study Report, MSL-20178.

The CP4 EPSPS protein produced in MON 89788 was characterised to determine that the expected protein was being produced. The *cp4 epsps* gene encodes a 47.7 kDa protein (calculated based on predicted amino acid sequence) consisting of a single polypeptide of 455 amino acids. The CP4 EPSPS protein in MON 89788 is translocated to the chloroplasts via an N-terminal fusion with CTP2 to form the CTP2-CP4 EPSPS precursor protein. This protein is then processed to remove the transit peptide, resulting in the mature functional CP4 EPSPS protein.

The majority of chloroplastic proteins are encoded in the nucleus and synthesised as precursor proteins that have a cleavable amino-terminal transit peptide which facilitates their post-translational import into the organelle. Following import, transit peptides are cleaved off by the stromal processing peptidase (SPP). The accumulation of transit peptides has been reported to have severe effects on the integrity and function of chloroplasts as they can insert into lipid bilayers and damage chloroplast membranes.

This necessitates their rapid removal by proteolytic degradation. Following cleavage of the transit peptide from the precursor protein to produce the mature protein, the transit peptide subfragment has been shown to be rapidly degraded by a metalloprotein (Richter and Lamppa, 2002). A zinc-metalloprotease that is targeted to both the mitochondria and chloroplast, and that proteolytically degrades both mitochondrial and chloroplastic targeting peptides has been isolated and characterised (Bhushan *et al*, 2003). This proteolytic activity against transit peptides was not sequence specific but appears to recognise and degrade unstructured peptides and is inactive against folded structures (Moberg *et al*, 2003). Therefore, it is considered unlikely that the CTP2 transit peptide would accumulate to any significant levels in soybean MON 89788.

The molecular identity and biochemical characteristics of the CP4 EPSPS protein expressed *in planta* were examined using a variety of biochemical techniques.

SDS-PAGE and Western blot analysis of the MON 89788 produced CP4 EPSPS protein revealed a protein with a molecular weight of approximately 44 kDa, which was consistent with the *E. coli* produced protein. This band was excised and N-terminal sequence analysis performed, the results of which indicated that the expected amino acid sequence of the mature CP4 EPSPS protein was present, with the exception of the initial methionine. As the DNA sequence demonstrated the presence of the methionine codon, the removal of the methionine in the purified plant-produced protein is likely due to cellular enzyme processing in the plant. This has been observed previously in canola, sugar beet, corn, cotton and soybean. The N-terminal sequence data confirms that the purified protein extracted from MON 89788 is the mature form of CP4 EPSPS and is consistent with the sequence of the *E. coli* produced reference standard.

MALDI-TOF mass spectrometry analysis identified 23 protein fragments that matched the expected mass of the trypsin-digested CP4 EPSPS protein. These covered 50.3% of the protein and identified it as the expected protein. A protein can usually be identified when 40% of the mass fragments are identified from the analysed protein. Immunoblot analysis with CP4 EPSPS specific antisera (goat antisera) also positively identified the approximately 44 kDa band as CP4 EPSPS and equivalent to the *E. coli* produced protein.

The functional activities of the plant-produced CP4 EPSPS protein and the *E. coli*-produced CP4 EPSPS reference standard were determined using a phosphate release assay. The specific activities of the MON 89788-produced CP4 EPSPS and *E. coli*-produced CP4 EPSPS proteins were 3.7 U/mg total protein and 4.4 U/mg total protein, respectively. Other studies have reported the average specific activity of the CP4 EPSPS protein to be between 3 and 6 U/mg. The enzyme assay demonstrated that the plant-produced CP4 EPSPS protein was as active as *E. coli*-produced CP4 EPSPS protein and thus the plant-produced protein is functionally equivalent to the *E. coli*-produced protein.

The isolated plant-produced CP4 EPSPS protein was analysed for post-translational modification through covalently bound carbohydrate moieties. After labelling with biotin, protein-bound carbohydrate moieties were detected with streptavidin-horseradish peroxidase and enhanced chemiluminescence. The *E. coli*-produced CP4 EPSPS protein was used as a non-glycosylated negative control and the transferrin protein as a positive control. There was a very faint band migrating at 44 kDa in both the plant and *E. coli* produced samples. This was considered to be non-specific reactivity as bacterial expression systems such as *E. coli* lack the ability to glycosylate proteins.

As the markers are non-glycosylated proteins produced in *E. coli*, the presence of a faintly hybridising band at 20 kDa in the marker lane supports this conclusion. Therefore this analysis suggests that MON 89788-produced CP4 EPSPS is equivalent to the *E. coli* produced protein.

A combination of N-terminal sequence analysis, MALDI-TOF and Western blot have confirmed the identity of the plant-produced CP4 EPSPS protein. The characterisation of the *E. coli*-produced CP4 EPSPS protein indicates it is equivalent to the plant-produced CP4 EPSPS protein based on comparable electrophoretic mobility, enzyme activity, immunoreactivity and absence of detectable glycosylation. Based on the similarity of the results from the plant and microbial preparations, the MON 89788-produced protein is chemically and functionally equivalent to CP4 EPSPS protein expressed in *E. coli*.

4.4 Potential toxicity of novel proteins

Studies submitted:
McCoy, R.L. and A. Silvanovich (2003) Bioinformatics Analysis of the CP4 EPSPS Protein Utilizing the AD4, TOXIN5 and ALLPEPTIDES Databases. Unpublished Monsanto Report MSL-18752.

McCoy, R.L. and A. Silvanovich (2005) Updated Bioinformatics Evaluation of the CP4 EPSPS Protein Utilizing the AD5 Database. Monsanto Study Report MSL-19894.

Leach J.N., R.E. Hileman, J.J. Thorp, C. George and J. Astwood. (2002) Assessment of the *in vitro* digestibility of purified *E. coli*-produced CP4 EPSPS protein in simulated gastric fluid. Unpublished Monsanto Study Report MSL-17566.

The mature CP4 EPSPS protein in glyphosate-tolerant soybean MON 89788 is biochemically similar to the EPSPS proteins naturally present in a variety of food crops (e.g. soybean and corn), which have a history of safe consumption by humans (Padgette *et al*., 1996; Harrison *et al.,* 1996). Also, the mature CP4 EPSPS protein in glyphosate-tolerant soybean MON 89788 is identical to, or shares greater than 99% sequence identity to, the amino acid sequence of the CP4 EPSPS protein produced in a number of other glyphosate-tolerant crops that have previously been approved for food use by FSANZ.

The CP4 EPSPS protein has previously undergone assessment by FSANZ when present in other GM (glyphosate-tolerant) crop varieties including soybean, cotton, canola, sugarbeet corn and lucerne. The data submitted for an assessment of potential toxicity have therefore been comprehensively appraised (see Final Assessment Reports for FSANZ Applications A338, A355, A362, A363, A378, A416, A525, A548, A553 and A575).

These assessments considered history of previous exposure to the protein through the diet, bioinformatic analysis of the primary and secondary structure of the CP4 EPSPS protein to examine any similarities with known protein toxins, biochemical tests (heat stability), and acute oral toxicity studies in animals. The previous assessments concluded that the CP4 EPSPS protein is not toxic and is therefore safe for human consumption.

Acute toxicity studies

To generate sufficient quantities of the CP4 EPSPS protein required for toxicity, and biochemical studies, it is necessary to produce the protein in bacterial expression systems. Prior to use, the bacterially produced protein is compared to the protein produced in the plant, to demonstrate their equivalence.

The CP4 EPSPS used for further analyses was produced in the laboratory using recombinant *E. coli*. As outlined in the previous section, a range of biochemical methods was used to establish that *E. coli*-produced CP4 EPSPS protein is equivalent to the protein produced by glyphosate-tolerant soybean MON 89788.

The acute toxicity of the CP4 EPSPS protein has been previously tested by acute gavage exposure in mice and no deleterious effects were observed (Harrison *et al.,* 1996). The CP4 EPSPS protein was administered at levels 1000 fold higher than those anticipated in consumption of food products; the no effect level (NOEL) for oral toxicity in mice is 572 mg/kg body weight, and was the highest dose tested. Despite this high dose, there was no mortality or morbidity, and there were no significant differences in terminal body weights of animals in the treated and control groups. Upon necropsy, body cavities were opened and organs examined *in situ* and removed. There were no pathological findings attributable to the treatment with the CP4 EPSPS protein.

4.5 Potential allergenicity of novel proteins

The potential allergenicity of a novel protein is evaluated using an integrated, step-wise, case-by-case approach relying on various criteria used in combination, since no single criterion is sufficiently predictive of either allergenicity or non-allergenicity. The assessment focuses on:

(i) the source of the novel protein,
(ii) any significant amino acid sequence similarity of the novel protein with that of known allergens, and
(iii) structural properties of the novel protein, including susceptibility to degradation in simulated digestion models.

In some cases, such as where the novel protein has sequence similarity to a known allergen, additional *in vitro* and *in vivo* immunological testing may be warranted. Applying such criteria systematically provides reasonable evidence on the potential of the novel protein to act as an allergen.

Source of protein

The CP4 EPSPS protein in soybean MON 89788 is derived from a naturally occurring, glyphosate-degrading bacterium, *Agrobacterium tumefaciens*, identified by the American Type Culture Collection. Species of *Agrobacterium* are not known human or animal pathogens, nor known to be allergenic.

Similarity to known allergens

Potential structural similarities between the CP4 EPSPS enzyme and proteins in the allergen databases (AD4 and AD5) were evaluated using the FASTA sequence alignment tool. Inspection of the results showed no significant similarities between the CP4 EPSPS protein and known allergens. The most significant alignment, to dust mite allergen Der f II, was only 30.5% identical over an 82 amino acid window requiring five gaps for alignment.

This is less than the threshold for considering the possibility of cross-reactivity of 35% identity across 80 or more amino acids suggested by (Codex, 2004). This alignment produced an E score2 of 0.66 and is not considered to indicate structural or functional homology between the CP4 EPSPS protein and the dust mite allergen Der f II. No immunologically relevant sequences (identity across eight contiguous amino acids) were detected when the CP4 EPSPS sequence was compared to the AD4 or AD5 sequence databases using a pair-wise comparison algorithm.

In vitro digestibility

Typically, food proteins that are allergenic tend to be stable to enzymes such as pepsin and the acidic conditions of the digestive system, exposing them to the intestinal mucosa and leading to an allergic response (Astwood and Fuchs, 1996; Metcalfe *et al*., 1996; Kimber *et al*., 1999). Novel proteins are therefore investigated for their digestibility in simulated digestion models.

Previous assessment of the CP4 EPSPS protein found that it is rapidly degraded in simulated digestive fluids. The half-life of CP4 EPSPS was less than 15 seconds in the gastric system and less than 10 minutes in the intestinal system, based on Western blot analysis (Harrison *et al.,* 1996). Subsequent experiments to assess the *in vitro* digestibility of the CP4 EPSPS protein in simulated gastric fluid (SGF) showed that 95-98% of the CP4 EPSPS protein was digested within 15 seconds. Similarly, the EPSPS activity was reduced to <10% within 15 seconds of incubation in SGF.

4.6 Analysis of potential ORFs within the insert and at the junction regions

Studies Submitted:
McClain, J.S. and A. Silvanovich (2006) Bioinformatics Evaluation of DNA Sequences Flanking the 5' and 3' Junctions of the Inserted DNA in MON 89788 Soybean. Assessment of Putative Polypeptides. Monsanto Company unpublished report. MSL-20344.

As part of a comprehensive safety assessment, bioinformatics analyses were performed to assess the similarity to known allergens, protein toxins or pharmacologically active proteins of the putative polypeptides encoded by the DNA spanning the junctions between soybean genomic DNA and the 5' and 3' ends of the inserted DNA. Sequences spanning either the 5' or 3' junction region were translated from stop codon to stop codon in all six reading frames. As mentioned in Section 3.4, two of the novel open reading frames between stop codons were less than eight amino acids, so bioinformatics analysis of the other ten putative open reading frames was performed using the ALLPEPTIDES, TOXIN5 and AD6 (the allergen database) databases.

No alignments with any of the query sequences generated an E score of less than 1×10^{-5}. In addition to structural similarity, each putative polypeptide was screened for short polypeptide (eight amino acids) matches using a pair-wise comparison algorithm.

[2] The E score reflects the degree of similarity between a pair of sequences and can be used to evaluate the significance of an alignment. The calculated E score depends on the overall length of joined (gapped) local sequence alignments, the quality (percent identity/similarity) of the overlap and the size of the database used for the FASTA search (Pearson and Lipman, 1988). For a pair of sequences, very small E score values may indicate a structurally relevant similarity. Conversely, large E score values are typically associated with poor alignments that do not represent a biologically relevant structural similarity.

No biologically relevant structural similarity to allergens, toxins, or bioactive proteins was observed for any of the potential polypeptides. A single eight amino acid alignment occurred between one of the sequences and an entry from the AD6 database, an unnamed protein from Indianmeal moth (*Plodia interpunctella*). However, as part of a recent review of all sequences in the AD6 database by an independent expert allergen panel, this protein sequence, along with 377 others, has been removed from subsequent versions of the database as there is insufficient evidence of allergenicity[3]. Excluding this result, there are no immunologically significant epitopes present in any of the reading frames at either DNA-insert junction.

The results of these bioinformatic analyses demonstrate that even in the unlikely event that the transgene junctions were transcribed, and further that any of the junction ORFs were translated, they would not share a sufficient degree of sequence similarity or identity to indicate that they would be potentially toxic, allergenic or have other health implications.

4.7 Conclusion

The CP4 EPSPS protein is expressed in MON 89788 soybean grain at a mean of 140 µg/g fresh weight.

The characterisation of the CP4 EPSPS protein in MON 89788 indicates it is chemically and functionally equivalent to the *E. coli*-produced CP4 EPSPS protein based on comparable electrophoretic mobility, enzyme activity, immunoreactivity and absence of detectable glycosylation. These studies further indicate that the CP4 EPSPS protein is expressed in soybean MON 89788 as expected and does not appear to have undergone any unexpected post-translational modification. Therefore, previous studies of the acute toxicity carried out using *E. coli*-produced CP4 EPSPS protein are applicable to the protein produced by glyphosate-tolerant soybean MON 89788. No deleterious effects of CP4 EPSPS protein were observed in the toxicity study.

The CP4 EPSPS protein is structurally and biochemically similar to other EPSPS enzymes from various plant food sources that are currently part of the human diet and have been consumed over a long period of time without health concerns. The potential toxicity and allergenicity of the CP4 EPSPS protein has been assessed by FSANZ on numerous occasions and no adverse findings have been reported. Its use is approved in food derived from specific lines of soybean, sugarbeet, corn, cotton, canola and lucerne.

5. COMPOSITIONAL ANALYSES

A comparison of similarities and differences in composition between a GM plant and its conventional counterpart aids in the identification of potential safety and nutritional issues and is considered the most appropriate strategy for the safety and nutritional assessment of GM foods (WHO, 2000). Ideally, the comparator should be the near isogenic parental line grown under identical conditions. In this case, the transgene is the only genetic difference between the two tested varieties. The composition of glyphosate-tolerant soybean MON 89788 was compared to that of the A3244 control, the parent soybean line used for the initial transformation. In addition, twelve different conventional soybean varieties were included as additional comparators to establish reference ranges for compositional constituents.

[3] http://www.allergenonline.com/about.asp

Any statistically significant differences between glyphosate-tolerant soybean MON 89788 and the control A3244 can be compared to the reference range to assess whether the differences are likely to be biologically relevant.

5.1 Key components

When determining similarities and differences in composition between a GM plant and its conventional counterpart, the critical components measured are determined by identifying key nutrients, key toxicants and anti-nutrients for the food source in question (FAO, 1996). The key nutrients and anti-nutrients are those components in a particular food that have a substantial impact in the overall diet. These can be major constituents (e.g., fats, proteins, carbohydrates) or minor components (e.g., minerals, vitamins). Key toxicants are those toxicologically significant compounds known to be inherently present in the plant, such as those compounds whose potency and level may be significant to health (e.g., increased levels of solanine in potatoes).

As a minimum, the key nutrients of soybean seed appropriate for a comparative study include the proximates (crude protein, fat, ash, acid detergent fibre and neutral detergent fibre), amino acids and fatty acids. In addition, international guidance suggests levels of the key anti-nutrients phytic acid, trypsin inhibitors, lectins and isoflavones should be determined for new varieties of soybean (OECD, 2001). Phytic acid chelates mineral nutrients (including calcium, magnesium, potassium, iron and zinc) making them unavailable to monogastric animals, including humans. Protease inhibitors interfere with digestion of protein. Lectins are proteins that bind to carbohydrate-containing molecules. Both protease inhibitors and lectins can inhibit growth. The activity of protease inhibitors and lectins is heat-labile and they are inactivated during processing of soybean protein products and soybean meal so that the final edible soybean product should contain minimal levels of these anti-nutrients. Soybean contains a number of isoflavones reported to possess biochemical activity including estrogenic, anti-estrogenic and hypercholesterolaemic effects that have been implicated in adversely affecting animal reproduction (OECD, 2001). The three basic types of isoflavones in soybeans are daidzein, genistein and glycitein. Soybean also contains two low molecular weight carbohydrates, stachyose and raffinose, that are considered to be anti-nutrients due to the gas production and resulting flatulence caused by their consumption (OECD, 2001).

5.2 Levels of Key components

Study submitted:
Lundry, D.R., S.G. Riordan, M.L. Breeze and R. Sorbet (2006) Amended report for MSL-20163: Composition Analyses of Soybean Forage and Seed Collected from MON 89788 Grown in the United States during the 2005 Field Season. Unpublished Monsanto study report MSL-20300.

Compositional analysis

Compositional analyses of the soybean seed included proximates (protein, fat, ash and moisture and carbohydrates by calculation), acid detergent fibre (ADF), neutral detergent fibre (NDF), amino acid composition, fatty acid composition (C8-C22), vitamin E, the anti-nutrients stachyose, raffinose, trypsin inhibitor, phytic acid, lectin and isoflavones (daidzein, genistein and glycitein). In all, 56 analytical components of soybean grain were measured according to established protocols.

Of the 56 components analysed, 14 minor fatty acids were excluded from the summary and analysis as more than half the observed values were below the assay's limit of quantitation.

Compositional analyses were conducted on soybean grown at five field sites across the United States during the 2005 growing season. Sites were located in York County (Nebraska), Clinton County (Illinois), Warren County (Illinois), Jackson County (Arkansas), and Fayette County (Ohio). Seed was planted in a randomised complete block design with three replicates of each test, control and reference substance. Plots containing glyphosate-tolerant soybean MON 89788 were treated with a commercial rate of glyphosate herbicide. One control seed sample and seven reference samples contained trace amounts of MON 89788 or control 40-3-2 (≤ 3.05%). These levels of contamination are considered too low to have significantly affected the results of the compositional analyses.

Statistical analyses of the compositional data were conducted using a mixed model analysis of variance method. The five sites were analysed both separately and combined, giving six sets of comparisons. Statistical evaluation of the composition data compared the seed from the soybean test population to the non-transgenic control population. Statistically significant differences were determined at the 5% level of significance ($p<0.05$). SAS® software was used to generate all summary statistics and perform all analyses.

Data from commercial varieties were not included in the final statistical analysis. The reference population data were used to develop population tolerance intervals. For each compositional component, 99% tolerance intervals were calculated that are expected to contain, with 95% confidence, 99% of the quantities expressed in the population of commercial lines.

In a study of this magnitude, a small percentage (approximately 5%) of statistically significant differences is expected to occur due to chance alone. For those comparisons in which the glyphosate-tolerant soybean test result was statistically different from the control, the test range was compared to the 99% tolerance interval derived from the commercial varieties. This determines whether the range of values for each test population is within the variance of a population of the commercial soybean varieties. Statistically significantly different values were also compared to literature ranges and ranges reported in the International Life Science Institute Crop Composition Database (ILSI, 2004).

Results and Discussion

The results of the combined site comparisons for grain are presented in Table 4. A summary of the statistically significant differences between glyphosate-tolerant soybean MON 89788 and the control line is presented in Table 5.

Results from the combined site analyses conducted on seed samples derived from glyphosate-tolerant soybean MON 89788 indicated that there were no statistically significant differences for 39 of the 42 analytes measured. There were three statistically significant differences observed between the test grain and non transgenic control: levels of the anti-nutrients daidzein and glycitein were lower in MON 89788 than in the control, and levels of vitamin E were higher in MON 89788 than the control.

For the means of the analytes that were statistically significantly different ($p<0.05$) from the control, the values for MON 89788 were within the 99% tolerance interval developed from the conventional soybean varieties grown at the same locations (Table 5). The mean levels are also within the ILSI and literature ranges (Table 6). Hence, these differences are unlikely to be biologically meaningful.

Analytes were also examined for reproducibility and trends across sites. Statistically significant differences were observed in as many as two sites for only one component, raffinose. The levels of raffinose in MON 89788 were lower than the control at one site (AR) and higher than the control at the other (IL-2). As there is no consistent trend and the values are within the 99% tolerance interval and literature and ILSI ranges, they are unlikely to be biologically relevant (Table 5). For 16 analytes, statistically significant differences were observed at only one site. As these differences were not reproducible across sites and are within the 99% tolerance interval for conventional soybeans grown across sites and the ILSI and literature ranges, the differences are not considered to be biologically relevant (Table 5). In a study of this magnitude, a small percentage (approximately 5%) of statistically significant differences is expected to occur due to chance alone. Differences occurring in one of the field sites only which are not repeated at other sites, are not indicative of a pattern of change that could be attributed to the genetic changes and are more likely to be random occurrences. In this comparative study, changes in the levels of some analytes are in this category. Consequently, these differences, although statistically significant for the individual site, are not considered to be biologically meaningful.

The compositional data are consistent with the conclusion that grain from soybean MON 89788 is compositionally equivalent to grain produced by the control soybean variety and to conventional soybean varieties currently on the market.

Table 4: Statistical Summary of Combined-Site Soybean Grain Key Components for test MON 89788 vs. A3244

Analytical Component (Units)[1]	MON 89788 Mean (S.E.) [Range]	A3244 Mean (S.E.) [Range]	Difference (MON 89788 minus A3244) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Conventional (Range) [99% Tol. Int.[2]]
Amino Acid (% DW)						
Alanine (% DW)	1.77 (0.017) [1.56 - 1.87]	1.77 (0.018) [1.71 - 1.83]	-0.0035 (0.018) [-0.19 - 0.069]	-0.042, 0.035	0.845	(1.62 - 1.89) [1.51, 2.00]
Arginine (% DW)	3.06 (0.082) [2.73 - 3.31]	3.07 (0.083) [2.76 - 3.34]	-0.0095 (0.037) [-0.26 - 0.33]	-0.090, 0.071	0.801	(2.61 - 3.27) [2.27, 3.60]
Aspartic Acid (% DW)	4.73 (0.068) [4.20 - 5.08]	4.72 (0.070) [4.42 - 4.98]	0.0072 (0.045) [-0.41 - 0.33]	-0.090, 0.10	0.875	(4.21 - 5.02) [3.85, 5.44]
Cystine (% DW)	0.62 (0.0084) [0.58 - 0.67]	0.62 (0.0085) [0.59 - 0.65]	-0.00028 (0.0050) [-0.044 - 0.026]	-0.011, 0.010	0.955	(0.57 - 0.65) [0.55, 0.67]
Glutamic Acid (% DW)	7.53 (0.12) [6.69 - 8.20]	7.49 (0.13) [6.97 - 7.90]	0.035 (0.075) [-0.63 - 0.53]	-0.13, 0.20	0.647	(6.62 - 8.19) [5.86, 8.96]
Glycine (% DW)	1.78 (0.020) [1.58 - 1.88]	1.78 (0.021) [1.71 - 1.86]	0.0012 (0.018) [-0.18 - 0.11]	-0.037, 0.040	0.949	(1.62 - 1.90) [1.46, 2.05]
Histidine (% DW)	1.07 (0.014) [0.95 - 1.13]	1.07 (0.015) [1.02 - 1.13]	-0.0035 (0.0099) [-0.10 - 0.057]	-0.025, 0.018	0.729	(0.96 - 1.13) [0.90, 1.21]
Isoleucine (% DW)	1.83 (0.029) [1.65 - 1.97]	1.83 (0.031) [1.70 - 1.99]	-0.0092 (0.030) [-0.22 - 0.26]	-0.071, 0.053	0.760	(1.64 - 2.00) [1.44, 2.16]

Table 4 (continued): Statistical Summary of Combined-Site Soybean Grain Key Components for test MON 89788 vs. A3244

Analytical Component (Units)[1]	MON 89788 Mean (S.E.) [Range]	A3244 Mean (S.E.) [Range]	Difference (MON 89788 minus A3244) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Conventional (Range) [99% Tol. Int.[2]]
Amino Acid (% DW)						
Leucine (% DW)	3.18 (0.040) [2.81 - 3.39]	3.18 (0.042) [3.04 - 3.33]	-0.0024 (0.031) [-0.32 - 0.20]	-0.070, 0.065	0.940	(2.89 - 3.42) [2.62, 3.66]
Lysine (% DW)	2.62 (0.025) [2.33 - 2.76]	2.62 (0.026) [2.51 - 2.73]	-0.00003 (0.023) [-0.25 - 0.13]	-0.051, 0.050	0.998	(2.40 - 2.77) [2.22, 2.95]
Methionine (% DW)	0.52 (0.0059) [0.47 - 0.56]	0.53 (0.0062) [0.50 - 0.55]	-0.0081 (0.0060) [-0.040 - 0.032]	-0.021, 0.0049	0.200	(0.45 - 0.56) [0.42, 0.60]
Phenylalanine (% DW)	2.10 (0.030) [1.84 - 2.24]	2.10 (0.031) [2.00 - 2.19]	-0.0011 (0.021) [-0.21 - 0.14]	-0.047, 0.045	0.959	(1.90 - 2.29) [1.70, 2.45]
Proline (% DW)	2.05 (0.029) [1.81 - 2.21]	2.05 (0.029) [1.95 - 2.16]	0.0047 (0.020) [-0.18 - 0.12]	-0.039, 0.048	0.819	(1.86 - 2.23) [1.66, 2.38]
Serine (% DW)	2.23 (0.029) [1.93 - 2.42]	2.21 (0.030) [2.08 - 2.28]	0.019 (0.023) [-0.16 - 0.17]	-0.031, 0.069	0.432	(1.99 - 2.42) [1.84, 2.54]
Threonine (% DW)	1.58 (0.014) [1.42 - 1.68]	1.59 (0.015) [1.51 - 1.66]	-0.0073 (0.013) [-0.13 - 0.062]	-0.035, 0.020	0.573	(1.44 - 1.67) [1.38, 1.76]
Tryptophan (% DW)	0.39 (0.015) [0.34 - 0.44]	0.39 (0.015) [0.33 - 0.46]	-0.0025 (0.015) [-0.10 - 0.064]	-0.044, 0.039	0.875	(0.30 - 0.47) [0.25, 0.54]

Table 4 (continued): Statistical Summary of Combined-Site Soybean Grain Key Components for test MON 89788 vs. A3244

Analytical Component (Units)[1]	MON 89788 Mean (S.E.) [Range]	A3244 Mean (S.E.) [Range]	Difference (MON 89788 minus A3244) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Conventional (Range) [99% Tol. Int.[1]]
Amino Acid (% DW)						
Tyrosine (% DW)	1.41 (0.019) [1.25 - 1.48]	1.42 (0.020) [1.33 - 1.47]	-0.0091 (0.015) [-0.12 - 0.070]	-0.051, 0.033	0.582	(1.28 - 1.51) [1.18, 1.64]
Valine (% DW)	1.91 (0.035) [1.73 - 2.05]	1.93 (0.036) [1.77 - 2.11]	-0.017 (0.032) [-0.24 - 0.28]	-0.084, 0.051	0.615	(1.71 - 2.09) [1.51, 2.27]
Fatty Acid (% DW)						
16:0 Palmitic (% DW)	2.07 (0.094) [1.84 - 2.40]	2.07 (0.094) [1.71 - 2.46]	-0.0027 (0.052) [-0.21 - 0.24]	-0.14, 0.14	0.961	(1.66 - 2.35) [1.32, 2.64]
18:0 Stearic (% DW)	0.78 (0.027) [0.65 - 0.89]	0.77 (0.027) [0.61 - 0.86]	0.012 (0.018) [-0.053 - 0.14]	-0.036, 0.060	0.531	(0.63 - 1.07) [0.37, 1.28]
18:1 Oleic (% DW)	3.53 (0.14) [3.05 - 4.24]	3.54 (0.14) [2.92 - 4.09]	-0.015 (0.10) [-0.40 - 0.51]	-0.29, 0.26	0.890	(2.99 - 5.29) [2.06, 6.43]
18:2 Linoleic (% DW)	9.17 (0.47) [8.00 - 10.42]	9.25 (0.47) [7.42 - 11.29]	-0.079 (0.21) [-0.86 - 0.99]	-0.64, 0.48	0.720	(8.41 - 10.69) [7.75, 11.22]
18:3 Linolenic (% DW)	1.29 (0.063) [1.09 - 1.48]	1.30 (0.063) [1.09 - 1.60]	-0.0059 (0.028) [-0.13 - 0.15]	-0.082, 0.070	0.843	(1.02 - 1.55) [0.84, 1.69]
20:0 Arachidic (% DW)	0.061 (0.0026) [0.049 - 0.071]	0.060 (0.0026) [0.046 - 0.068]	0.0012 (0.0016) [-0.0048 - 0.012]	-0.0031, 0.0055	0.482	(0.046 - 0.076) [0.031, 0.094]

Table 4 (continued): Statistical Summary of Combined-Site Soybean Grain Key Components for test MON 89788 vs. A3244

Analytical Component (Units)[1]	MON 89788 Mean (S.E.) [Range]	A3244 Mean (S.E.) [Range]	Difference (MON 89788 minus A3244) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Conventional (Range) [99% Tol. Int.[2]]
Fatty Acid (% DW)						
20:1 Eicosenoic (% DW)	0.042 (0.0031) [0.032 - 0.050]	0.042 (0.0031) [0.029 - 0.053]	0.00036 (0.0013) [-0.0062 - 0.0073]	-0.0032, 0.0039	0.796	(0.030 - 0.057) [0.021, 0.065]
22:0 Behenic (% DW)	0.063 (0.0030) [0.050 - 0.072]	0.062 (0.0031) [0.046 - 0.071]	0.00094 (0.0014) [-0.0056 - 0.0096]	-0.0029, 0.0048	0.539	(0.046 - 0.073) [0.034, 0.091]
Fiber						
Acid Detergent Fiber (% DW)	18.01 (0.94) [14.64 - 23.94]	17.46 (0.95) [14.39 - 22.44]	0.54 (1.21) [-3.22 - 5.67]	-2.79, 3.88	0.676	(13.30 - 26.26) [9.62, 28.57]
Neutral Detergent Fiber (% DW)	18.18 (0.46) [16.38 - 20.49]	19.11 (0.48) [15.60 - 20.73]	-0.93 (0.60) [-3.35 - 2.77]	-2.34, 0.49	0.165	(14.41 - 23.90) [13.26, 26.33]
Isoflavones						
Daidzein (ug/g DW)	993.67 (114.34) [631.32 - 1571.41]	1073.57 (114.79) [747.53 - 1526.23]	-79.90 (30.47) [-272.18 - 106.63]	-146.14, -13.66	0.021	(274.88 - 1485.52) [0, 1925.63]
Genistein (ug/g DW)	797.90 (49.93) [565.26 - 996.66]	824.83 (50.35) [651.01 - 1003.02]	-26.93 (19.52) [-151.16 - 74.36]	-69.66, 15.81	0.193	(354.09 - 984.29) [0, 1387.95]
Glycitein (ug/g DW)	91.77 (9.88) [53.78 - 162.52]	102.61 (10.01) [72.93 - 148.31]	-10.84 (4.69) [-32.97 - 30.19]	-20.98, -0.70	0.037	(52.72 - 298.57) [0, 287.45]

Table 4 (continued): Statistical Summary of Combined-Site Soybean Grain Key Components for test MON 89788 vs. A3244

Analytical Component (Units)[1]	MON 89788 Mean (S.E.) [Range]	A3244 Mean (S.E.) [Range]	Difference (MON 89788 minus A3244) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Conventional (Range) [99% Tol. Int.[2]]
Proximate						
Ash (% DW)	5.04 (0.12) [4.66 - 5.60]	5.03 (0.12) [4.75 - 5.46]	0.0099 (0.073) [-0.81 - 0.42]	-0.14, 0.16	0.892	(4.61 - 5.57) [4.00, 6.08]
Carbohydrates (% DW)	37.07 (0.54) [35.01 - 40.24]	36.88 (0.56) [35.17 - 40.74]	0.20 (0.55) [-2.38 - 2.95]	-1.30, 1.69	0.738	(32.75 - 40.98) [27.86, 45.79]
Fat (% DW)	17.57 (0.74) [15.35 - 19.98]	17.72 (0.74) [14.40 - 20.91]	-0.15 (0.42) [-1.74 - 1.73]	-1.28, 0.99	0.745	(15.97 - 20.68) [15.38, 21.95]
Moisture (% FW)	7.76 (0.47) [6.41 - 9.35]	7.51 (0.47) [6.51 - 9.63]	0.25 (0.27) [-0.44 - 1.31]	-0.51, 1.01	0.417	(6.24 - 9.11) [4.64, 9.94]
Protein (% DW)	40.32 (0.72) [37.31 - 42.54]	40.38 (0.73) [36.96 - 42.44]	-0.069 (0.31) [-1.72 - 2.44]	-0.74, 0.60	0.828	(36.48 - 43.35) [31.50, 47.45]
Vitamin						
Vitamin E (mg/100g DW)	2.71 (0.22) [1.88 - 3.72]	2.52 (0.22) [1.58 - 3.07]	0.19 (0.065) [-0.23 - 0.66]	0.043, 0.33	0.015	(1.29 - 4.80) [0, 7.00]
Antinutrient						
Lectin (H.U./mg FW)	4.29 (0.97) [0.70 - 9.77]	4.55 (1.01) [1.44 - 10.87]	-0.26 (1.02) [-8.11 - 5.75]	-2.38, 1.86	0.800	(0.45 - 9.95) [0, 9.72]
Phytic Acid (% DW)	0.76 (0.035) [0.58 - 0.93]	0.75 (0.037) [0.51 - 1.07]	0.011 (0.044) [-0.24 - 0.30]	-0.084, 0.11	0.811	(0.41 - 0.96) [0.39, 1.07]

Table 4 (continued): Statistical Summary of Combined-Site Soybean Grain Key Components for test MON 89788 vs. A3244

Analytical Component (Units)[1]	MON 89788 Mean (S.E.) [Range]	A3244 Mean (S.E.) [Range]	Difference (MON 89788 minus A3244) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Conventional (Range) [99% Tol. Int.[2]]
Raffinose (% DW)	0.52 (0.063) [0.40 - 0.71]	0.54 (0.063) [0.31 - 0.83]	-0.014 (0.041) [-0.20 - 0.11]	-0.13, 0.099	0.751	(0.26 - 0.84) [0, 1.01]
Antinutrient						
Stachyose (% DW)	2.36 (0.070) [2.02 - 2.85]	2.50 (0.073) [2.12 - 3.04]	-0.15 (0.10) [-0.59 - 0.53]	-0.38, 0.085	0.183	(1.53 - 2.98) [1.19, 3.31]
Trypsin Inhibitor (TIU/mg DW)	33.69 (2.84) [24.59 - 53.85]	31.44 (2.88) [23.43 - 41.91]	2.25 (1.56) [-4.81 - 13.99]	-2.32, 6.81	0.231	(20.79 - 55.51) [5.15, 59.34]

[1]DW = dry weight; FW = fresh weight; FA = fatty acid; S.E. = standard error; CI = Confidence Interval.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial varieties. Negative limits were set to zero.

Table 5: Summary of statistically significant differences comparing soybean MON 89788 to the Control A3244

Analytical Component (Units)[a]	Mean MON 89788 Test Event	Mean A3244 Control	Mean Diff. (% of A3244 Control Value)	Significance (p-Value)	MON 89788 Test Event (Range)	99% Tolerance Interval[c]
Statistical Differences Observed in Combined-Site Analyses						
Seed Daidzein (µg/g DW)	993.67	1073.57	-7.44	0.021	631.32 - 1571.41	0, 1925.63
Seed Glycitein (µg/g DW)	91.77	102.61	-10.56	0.037	53.78 - 162.52	0, 287.45
Seed Vitamin E (mg/100g DW)	2.71	2.52	7.41	0.015	1.88 - 3.72	0, 7.00
Statistical Differences Observed in More than One Site and Not in the Combined-Site						
Site AR[b] Seed Raffinose (% DW)	0.65	0.81	-20.02	0.024	0.58 - 0.71	0, 1.01
Site IL-2[b] Seed Raffinose (% DW)	0.42	0.33	25.45	.0.35	0.40 - 0.43	0, 1.01
Statistical Differences Observed in One Site and Not in the Combined-Site						
Site AR Seed Phenylalanine (% DW)	2.00	2.01	-0.41	0.014	2.00 - 2.01	1.70, 2.45
Site AR Seed 16:0 Palmitic (% DW)	2.21	2.40	-7.73	0.004	2.17 - 2.25	1.32, 2.64
Site AR Seed 18:0 Stearic (% DW)	0.76	0.81	-5.43	0.024	0.75 - 0.77	0.37, 1.28
Site AR Seed 18:1 Oleic (% DW)	3.30	3.68	-10.31	0.001	3.24 - 3.36	2.06, 6.43
Site AR Seed 18:2 Linoleic (% DW)	10.27	11.02	-6.86	0.005	10.06 - 10.42	7.75, 11.22
Site AR Seed 18:3 Linolenic ((% DW)	1.45	1.55	-6.16	0.029	1.41 - 1.48	0.84, 1.69
Site AR Seed 20:0 Arachidic (% DW)	0.060	0.064	-6.35	0.021	0.058 - 0.060	0.031, 0.094
Site AR Seed 20:1 Eicosenoic (%	0.048	0.053	-8.60	0.032	0.047 - 0.049	0.021, 0.065
Site AR Seed 22:0 Behenic (% DW)	0.066	0.070	-5385	0.034	0.064 - 0.068	0.034, 0.091
Site AR Seed ADF (% DW)	21.17	16.10	31.47	0.003	19.28 - 23.94	9.62, 28.57
Site AR Seed Carbohydrates (% DW)	38.13	36.02	5.88	0.048	37.77 - 38.42	27.86, 45.79
Site AR Seed Fat (% DW)	18.82	20.41	-7.79	0.002	18.42 - 19.17	15.38, 21.95
Site AR Seed Stachyose (% DW)	2.32	2.83	-18.13	0.010	2.10 - 2.50	1.19, 3.31
Site IL-2 Seed Genistein (µg/g DW)	762.46	849.88	-10.29	0.032	721.05 - 797.84	0, 1387.95
Site IL-2 Seed Moisture (% FW)	8.54	7.48	14.04	0.045	8.19 - 9.13	4.64, 9.94
Site NE[b] Seed NDF (% DW)	17.42	19.91	-12.51	0.023	16.79 - 18.39	13.26, 26.33

[a]DW = dry weight; FW = fresh weight. [b] AR = Arkansas Site; IL-2 = Warren county, Illinois Site; NE = Nebraska Site.
[c]With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

Table 6: Literature and ILSI Ranges for Components in Soybean Grain

Tissue/Component[1]	Literature Range[2]	ILSI Range[3]
Proximates (% DW)		
Ash	4.61-5.94[b]; 4.29-5.88[a]	3.885-6.542
Carbohydrates	29.3-41.3[a]	29.6-50.2
Fat, total	198-277[c] g/kg DW; 160-231[d] g/kg DW	8.104-23.562
Moisture (% FW)	5.3-8.73[a], 5.18-14.3[b]	5.1-14.9
Protein	329-436[c] g/kg DW; 360-484[d] g/kg DW	33.19-45.48
Fiber (% DW)		
Acid detergent fiber (ADF)	not available	7.81-18.61
Neutral detergent fiber (NDF)	not available	8.53-21.25
Crude fiber	5.74-7.89[a]	4.12-10.93
Amino Acids (%DW)	**% Dw[a]**	**% DW[h]**
Alanine	1.60— 1.86	1.513-1.851
Arginine	2.56—3.46	2.285-3.358
Aspartic acid	4.18 —4.99	3.808-5.122
Cystine/Cysteine	0.54 — 0.66	0.370-0.808
Glutamic acid	6.64—8.16	5.843-8.093
Glycine	1.60 - 1.87	1.458-1.865
Histidine	0.98— 1.16	0.878-1.175
Isoleucine	1.65 — 1.95	1.563-2.043
Leucine	2.81 — 3.37	2.590-3.387
Lysine	2.47 — 2.84	2.285-2.839
Methionine	0.51 — 0.59	0.443-0.668
Phenylalanine	1.78 — 2.19	1.632-2.236
Proline	1.86—2.23	1.687-2.284
Serine	1.96—2.28	1.632-2.484
Threonine	1.51—1.73	1.251-1.618
Tryptophan	0.56 — 0.63	0.356-0.501
Tyrosine	1.35—1.59	1.016-1.559
Valine	1.71 —2.02	1.627-2.204
Tissue/Component[1]	**Literature Range[2]**	**ILSI Range[3]**
Fatty Acids (% DW)		
12:0 Lauric	not available	not available
14:0 Myristic	not available	not available
16:0 Palmitic	1.44-2.31[f]	not available
16:1 Palmitoleic	not available	not available
17:0 Heptadecanoic	not available	not available
17:1 Heptadecenoic	not available	not available
18:0 Stearic	0.54-0.91[f]	not available
18:1 Oleic	3.15-8.82[f]	not available
18:2 Linoleic	6.48-11.6[f]	not available
18:3 Linolenic	0.72-2.16[f]	not available
20:0 Arachidic	0.04-0.7[f]	not available
20:1 Eicosenoic	not available	not available
20:2 Eicosadienoic	not available	not available
22:0 Behenic	not available	not available

Table 6 (continued): Literature and ILSI Ranges for Components in Soybean Grain

Vitamins (mg/100 g)	**FW[i]**	**DW**
Vitamin E	0.85g	0.47-6.17
Anti-Nutrients		
Lectin (H.U./mg FW)	0.8-2.4[a]	0.105-9.038
Trypsin Inhibitor (TIU/mg DW)	33.2-54.5[a]	19.59-118.68
Raffinose	not available	0.212-0.661
Stachyose	not available	1.21-3.50
Isoflavones	**mg/100 g FW**	**(mg/kg DW)**
Daidzein	9.88-l24.2[e]	60.0-2453.5
Genistein	13-150.1 [e]	144.3-2837.2
Glycitein	4.22-20.4[e]	15.3-310.4

[1]FW=fresh weight; DW=dry weight;
[2]Literature range references: [a](Padgette *et al*., 1996). [b](Taylor *et al*., 1999).
[c](Maestri *et al*., 1998). [d](Hartwig and Kilen, 1991). [e](USDA-ISU, 2002). [f](OECD, 2001).
[g](USDA, 2005). [h]Data converted from mg/g DW to g/l00g DW (% DW).
[i]Moisture value = 8.54g/100g.
[3]ILSI Soybean Database, 2004 (ILSI 2004).
Conversions: % DW x $l0^4$ = µg/g DW; mg/g DW x 10^3 = mg/kg DW;
mg/l00g DW X 10 = mg/kg DW; g/l00g DW x 10 = mg/g DW

5.3 Assessment of endogenous allergenic potential

Studies Submitted:
Rice, E.A. and G.A. Bannon (2006) Assessment of Human IgE Binding to Glyphosate-Tolerant Second Generation Soybean MON 89788, Control, and Reference Soy Extracts. Monsanto Company unpublished report. MSL-20552.

Soybean naturally contains allergenic proteins and is one of a group of known allergenic foods including milk, eggs, fish, shellfish, wheat, peanuts, tree nuts and sesame. This group of foods accounts for approximately 90% of all food allergies. The presence of allergenic proteins in the diet of hypersensitive individuals can cause severe adverse reactions. The allergenic effect of soybeans is attributed to the globulin fraction of soybean proteins that comprise about 85% of total protein (OECD, 2001). Soybean-allergic individuals will also be allergic to MON 89788 soy.

In order to assess whether MON 89788 has altered endogenous allergenic potential, a study was conducted to determine binding levels of IgE antibody to protein extracts prepared from MON 89788 and the parental soybean A3244. Extracts from 24 commercial varieties of soybean were also measured to provide a reference range.

Sera from 26 clinically documented, soybean-allergic individuals and six non-allergic individuals were used to assess the range of IgE binding to each soybean extract. The soybean allergic patients all had a documented history of anaphylactic reactions to soybean and a positive Double-Blind Placebo Controlled Food Challenge (DBPCFC). Aqueous extracts were prepared from the ground seeds of MON 89788, A3244 and the reference varieties, and analysed with a validated enzyme linked immuno-sorbent assay (ELISA) for IgE binding. The tolerance interval of each serum was established by the IgE binding values of the 24 commercial soybean extracts.

The tolerance interval represents the range of IgE binding to the commercial soybean varieties such that 99% of the IgE binding values are expected to fall within this range with 95% confidence.

Of the 26 sera from soy-allergic patients tested, 16 yielded positive IgE antibody binding values by ELISA for the majority of soy extracts. The lack of soy-specific IgE response in clinically confirmed soy allergic patients has been observed previously. None of the soybean varieties showed binding with the sera from non-allergic individuals.

For the 16 sera that yielded positive IgE values, the IgE-binding values of MON89788 and A3244 were compared to the calculated tolerance intervals. The results indicate that all MON 89788 and A3244 IgE binding values are within the established tolerance intervals for each serum, with the exception of one sample, where the IgE binding with A3244 was below the assay's limit of detection.

These data indicate that MON 89788 has similar IgE binding values to A3244 that are within the range established by the commercial soybean varieties. Thus, the levels of endogenous soybean allergens in MON 89788 and the control A3244 are comparable to the levels of endogenous allergens in commercially available soybean varieties.

5.4 Conclusion

Levels of key nutrients and key anti-nutrients in glyphosate-tolerant soybean MON 89788 were compared to levels in the non-transgenic parental line A3244 and to a range of conventional soybean varieties. The comparative analyses do not indicate any compositional differences of biological significance in the grain derived from glyphosate-tolerant soybean MON 89788 compared to the non-genetically modified control when grown in a range of geographical regions. With respect to both key nutrients and key anti-nutrients, soybean MON 89788 is compositionally equivalent to conventional soybean varieties. In addition, MON 89788 IgE binding to sera from soybean-allergic patients was within the tolerance interval established from 24 commercial soybean varieties and soybean MON 89788 is unlikely to have any greater allergenic potential than conventional soybean varieties.

6. NUTRITIONAL IMPACT

Establishing that a GM food is safe for human consumption is generally achieved through an understanding of the genetic modification and its direct consequences in the plant, together with an extensive compositional analysis of the food components derived from the GM plant and the non-GM counterpart.

To date, all approved GM plants with modified agronomic production traits (e.g. herbicide tolerance) have been shown to be compositionally equivalent to their conventional counterparts. Feeding studies in animals using feeds derived from the approved GM plants have shown equivalent nutritional performance to that observed with the non-GM feed. Thus the evidence to date is that where GM varieties have been shown to be compositionally equivalent to conventional varieties, feeding studies using target livestock species contribute minimally to a safety assessment.

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

In the case of glyphosate-tolerant soybean MON 89788, the extent of the compositional and other available data is considered sufficient to establish the nutritional adequacy of the food. However, a 42 day feeding study in broiler chickens was submitted by the Applicant and was therefore evaluated by FSANZ as additional supporting information.

6.1 Feeding study in chickens (42-days)

Study submitted:
Davis, S.W. (2006) Comparison of Broiler Performance and Carcass Parameters When Fed Diets Containing Soybean Meal Produced from MON 89788, Control or Reference Soybeans. Unpublished Monsanto Study No. 06-01-30-12.

Study aim

To assess the nutritional wholesomeness of diets containing soybean meal produced from MON 89788 in comparison to conventional soybean meal.

Study conduct

Ross x Ross 308 male and female broilers were used in a 42-day study to compare the feeding value of soybean MON 89788 to the parental soybean A3244, and reference soybean varieties (A2804, A3559, A4324, ST3870, A2824 and A3469). 800 birds were used; 100 (50 male, 50 female) birds for each of eight treatments.

Diets were formulated to be isocaloric and contain the maximum amount of soybean meal possible while remaining nutritionally adequate (approximately 33% for starter diets and 30% for grower/finisher diets). Feed and water were available *ad libitum* throughout the study.

Broilers were weighed by pen on days 0 and 42, and individually at study termination (day 43, 44 or 45). Feed intake per pen was determined for the 42 day period, allowing calculation of feed efficiency by pen, based on total weight of surviving broilers in the pen or adjusted to include weight gain of any broilers that died or were culled during the study. At study termination, all surviving birds were processed to determine carcass yield and meat composition. Fat pad measurements were taken for each bird. One broiler per pen was randomly selected for breast and thigh meat quality assays.

Results

Chick mortality was very low (1% of 960 chicks started on day 0). Mortality averaged across male and female birds from day 7 to 42 was also low and ranged between 1 -5%. MON 89788 treated birds had an average mortality rate of 4%. The mortality was random, without any relationship to treatment and was comparable to the rate commonly observed in chicks in commercial feeding trials.

Performance measures were not different (P>0.05) between the broilers fed diets containing MON 89788 and those fed control soybean meal with similar genetic background. These measurements included day 42 live bird weight, total feed intake, and unadjusted and adjusted feed conversion.

Likewise, carcass measurements were not different (P> 0.05) between birds fed MON 89788 diets and those on diets containing conventional soybean meal. These measurements included pre-processing live weight, chill weight, and weights of fat pad, breast, wing, drum and thigh parts. Moisture, protein and fat in the thigh and breast meat samples were similar between treatments.

For certain parameters, a significant (P>0.15) difference was observed between male and female birds. In these cases, males and females were analysed separately. In all cases, the diet containing MON 89788 produced results similar to the control or reference diets.

Conclusion

No unexpected effects on bird performance or health were observed in the birds fed MON 89788 soybean meal. The MON 89788 soybean diet was comparable to conventional soybean meal diets in terms of performance and carcass measurements.

7. OTHER STUDIES

In the case of glyphosate-tolerant soybean MON 89788, the extent of the molecular, compositional and other available data is considered sufficient to establish the safety of the food. However, the Applicant has also provided the results of a 90-day feeding study in rats with processed meal from MON 89788. While FSANZ does not routinely require animal toxicity studies to be undertaken, where such studies already exist, FSANZ will evaluate them as additional supporting information.

This approach is consistent with the recommendations of an expert panel FSANZ convened to consider the role of animal feeding studies in the safety assessment of genetically modified foods[4]. The panel noted that whole-food animal feeding studies may be informative in some limited circumstances, but that any potential adverse health effects can generally be identified by a scientifically informed comparative assessment of the GM food against its conventional counterpart. The panel also recommended that, where the results of relevant animal feeding studies are available, FSANZ evaluate them with critical attention to the methodology and potential limitations in interpretation of the results.

Study submitted:
A 90-day feeding study in rats with processed meal from MON 89788. (2007) Unpublished Monsanto Study No. MSL0020504.

Study aim

To evaluate the potential health effects of processed soybean meal from MON 89788 when fed to rats for at least 90 days.

[4] The workshop report is available at http://www.foodstandards.gov.au/foodmatters/gmfoods/roleofanimalfeedings3717.cfm

Study conduct

The study design was based on the OECD Guidelines for Testing of Chemicals, Health Effect Test Guidelines, Section 408, 21 September 1998 (OECD Guideline 408)[5].

Three groups of Sprague-Dawley rats, each consisting of 20 animals/sex/group, were used in a 90 day feeding study of a standard feed for rats formulated to contain approximately 15% (w/w) of soybean meal. The diets were formulated to conform to the specifications for PMI Certified Rodent LabDiet #5002, which contains approximately 15% (w/w) soybean meal. The control group received a diet formulated to contain approximately 15% (w/w) of meal from the control line A3244. One test group was administered a diet containing approximately 5% (w/w) of soybean meal from MON 89788, supplemented with approximately 10% (w/w) of soybean meal from the control line A3244. The second group received a diet formulated to contain approximately 15% (w/w) of meal from MON 89788.

Parameters Evaluated

All animals were observed twice daily for mortality and moribundity. Clinical examinations were performed daily and all significant readings were recorded. Detailed physical examinations, including behavioural observations were conducted weekly.

Individual body weights were recorded approximately weekly, beginning at least two weeks prior to administering the test or control diets. Mean body weights and mean cumulative body weight changes were calculated for each study week. Final body weights were recorded prior to scheduled necropsy.

Individual food consumption was recorded approximately weekly, beginning at least two weeks prior to administering the test or control diets. Food intake was calculated as g/animal/day. The mean amounts of test substance consumed (mg/kg/day) in the diets by each sex of each diet group was calculated based on the appropriate target concentration of test substance in the food (mg/kg of diet) and the mean food consumed (g/kg body weight/day).

Blood and urine samples were collected from 10 animals/sex/group on the day of scheduled necropsy during study week 13. These samples were used for clinical pathology evaluations (haematology, serum chemistry and urinalysis).

A complete necropsy was conducted on all animals, including examination of the external surface, all orifices, the cranial, thoracic, abdominal and pelvic cavities, including viscera. Tissues and organs specified in OECD Guideline 408 were collected and fixed. Organs designated in OECD Guideline 408 (except uterus and in addition thyroid) were weighed.

After processing into paraffin blocks, sectioning at 4-8 microns, mounting and staining with haematoxylin and eosin, any gross lesions present and the following tissues from all animals in the control and high-dose test groups were examined microscopically:

- adrenal glands (2);

[5] OECD Guidelines for the Testing of Chemicals are described and available at http://www.oecd.org/document/40/0,3343,en_2649_34377_37051368_1_1_1_1,00.html

- brain (representative regions including cerebrum, cerebellum and medulla/pons); epididymides (2);
- gastrointestinal tract (stomach, duodenum, jejunum, ileum, colon and rectum);
- heart; kidneys (2);
- liver (sections of two lobes);
- mesenteric lymph nodes;
- ovaries;
- pancreas;
- peripheral nerve (sciatic);
- spinal cord (cervical, mid-thoracic, lumbar);
- spleen; testes (2);
- thymus; and
- thyroid.

Statistical analyses were conducted using two-tailed tests (except as noted) comparing each test substance treated group to the control group by sex. Body weight, body weight change, food consumption, clinical pathology and organ weight data were subjected to a parametric one-way analysis of variance (ANOVA) to determine intergroup differences. Microscopic findings were compared using Fischer's exact test.

Results

All animals survived to the scheduled necropsy. The clinical findings recorded for animals in the test substance treated groups were noted with similar frequency in the control group, or were seen in isolated instances. None of the clinical findings were attributed to treatment because none were noted in a dose-related manner or they were common findings for laboratory rats of this age and strain.

Body weights and body weight changes were not adversely affected by administration of the test substance. There was a slight increase in the mean cumulative body weight gain for females in the 15% MON 89788 group during week 0 to 2 which was not considered toxicologically relevant because this increase in body weight gain did not persist and the cumulative weight change was generally the same in all female groups from weeks 0 to 13. Changes in body weights over the course of the study were similar for all groups of both males and females.

There were no statistically significant differences in food consumption between the control and test substance treated groups. The average consumption of MON 89788 over the duration of the study was 3,485 and 4,021 mg/kg bw/day for males and females in the 5% MON 89788 test group, and 10,490 and 12,066 mg/kg bw/day for males and females in the 15% MON 89788 test group.

There were no test substance related changes in haematology or urinalysis noted. The only statistically significant differences in serum chemistry between the control and test substance treated groups were in triglyceride and calcium levels, but these were not interpreted as being test substance related.

The mean triglyceride level for males in the 5% MON 89788 group (88 mg/dL) was statistically significantly higher than the control group (63 mg/dL) while the level for males in the 15% MON 89788 group (64 mg/dL) was virtually the same as the control mean. Closer analysis of the values for individual animals within the 5% MON 89788 diet group showed that the higher mean value was primarily due to four values that ranged between 105 and 142 mg/dL. These values above 100 mg/dL were interpreted to represent the upper range values for rats of this sex, age, strain and source, as they were found with similar frequency in a concurrent reference control study that used rats from the same shipment as those in this study. In the reference control study, rats were fed six different reference diets made with non-GM soybeans of different backgrounds. Of the 120 samples collected for triglyceride analysis, five males and four females had levels above 100 mg/dL (range 103 to 193 mg/dL). In addition, the mean triglyceride value in the male 5% MON 89788 diet group was within the range recorded in historical control data for the same strain of rats from subchronic studies. As the triglyceride values above 100 mg/dL appear to represent the upper range values for these rats and as the high values were not dose-related, they were not considered to be test-substance related.

Selected Serum Chemistry Values for Males

	Control and Test WIL-50296			Reference Population			
	Control diet Mean +/- SD	5% test diet Mean +/- SD	15% test diet Mean +/- SD	N	Population mean +/- SD	Min.	Max
Triglyceride (mg/dL)	63 +/- 15.7	88 +/- 31.5	64 +/- 12.6	60	70 +/- 19.8	35	125

One female in the 5% MON 89788 diet group had a markedly higher triglyceride value of 397 mg/dL, which was accompanied by higher alanine aminotransferase and cholesterol and lower chloride. The serum specimen was noted to be moderately lipemic. As the findings occurred in a single animal at the lowest dose level, they are interpreted as likely to be due to a spontaneous disease process not related to treatment with the test diet. The remaining females in the 5% MON 89788 diet group had triglyceride levels within normal limits.

The mean calcium level of females in the 15% test group was significantly lower than the control group mean but was within the range recorded for calcium levels in the concurrent reference control study and historical control data for this strain of rats in subchronic studies. Because of this, and the low magnitude of the difference, the difference was not considered to be test substance related.

Selected Serum Chemistry Values for Females

	Control and Test WIL-50296			Reference Population			
	Control diet Mean +/- SD	5% test diet Mean +/- SD	15% test diet Mean +/- SD	N	Population mean +/- SD	Min.	Max
Calcium (mg/dL)	10.9 +/- 0.24	10.9 +/- 0.38	10.6 +/- 0.33	60	11.1 +/- 0.35	10.3	12.1

In the anatomic pathology analysis, there were no test substance related macroscopic or microscopic findings at the scheduled necropsy, with all findings noted considered to be spontaneous and/or incidental in nature.

No test substance related effects on organ weights were detected, although brain weight relative to final body weight was statistically significantly lower in males in the 5% test diet group compared to the control.

As there was no concomitant change in males in the 15% MON 89788 diet group, the finding was not considered to be dose related. The mean male brain weight relative to final body weight was within the range recorded in the concurrent reference control study and historical control data for this strain of rats in subchronic studies.

Conclusion

There were no unscheduled deaths and no test substance related clinical observations. There were no test substance related effects on body weights, food consumption or haematology, serum chemistry or urinalysis parameters or on organ weights, macroscopic or microscopic findings.

The results support the conclusion that administration of soybean meal from MON 89788 at concentrations up to 15% in the diet (equivalent to 10.5 g/kg/day for males and 12.1 g/kg/day for females) for at least 90 days had no adverse effects on the growth or health of Sprague-Dawley rats.

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Attachment 3

Summary of first round of public consultation

Submitter	Option	Comments
Australian Food and Grocery Council	-	• Supports the Application, contingent upon satisfactory safety assessment by FSANZ. • Notes that an earlier version of glyphosate-tolerant soybean is already approved for food use and do not anticipate that there would be any health or safety concerns with this application.
Department of Human Services Victoria	-	• No objection to the Application progressing to Draft Assessment
Food Technology Association of Victoria Inc.	2	• No comment
New Zealand Food Safety Authority	-	• No comment at this stage. Will review the Draft Assessment Report
NSW Food Authority	-	• Supports the Application proceeding to Draft Assessment. • Notes that there are costs incurred in monitoring for the presence of GM Food. • Notes that The Director-General of the NSW Food Authority wrote to FSANZ on the cost impact of GM applications in April 2005. • Considers a national enforcement strategy surrounding GM food approvals should be developed.
Queensland Health	-	• No comment at this stage, but will review Draft Assessment Report when available

Summary of second round of public consultation

Submitter	Option	Comments
Private (Ivan Jeray)	1	• Believes GM foods have not been proven safe or economically viable and contaminate the food supply and the environment. • Notes that GM foods may not require labelling and believes consumers have a right to know what they will eat. Notwithstanding total opposition to application, believes all GM ingredients should require prominent labelling with print no smaller than size 12 font. • Protests at FSANZ's non-disclosure of GM food within application titles and believes all titles within the notification circular should clearly indicate the use of GM food.

Submitter	Option	Comments
Private (Penelope Gordon)	1	• If GM soy is approved, wants to know which products contain GM oils. Believes labelling requirements for GM and ingredients does not provide sufficient information to allow choice. • Notes that blended oils with labels stating 'vegetable oils' could be any combination of oils, making it difficult to avoid soy or canola oils, which may be derived from GM plants. • Notes that labels stating 'Made from Australian and imported ingredients' does not specify the proportion or identify the country the imported ingredients are from. • Would prefer that Australia completely avoid GM and believes consumers do not want GM products. • Believes all manufacturers and producers of foods should label their products with transparency and clarity.
Food Technology Association of Victoria Inc.	2	• FTA Victoria endorses the comments of the Technical Sub Committee: The committee accepted Option 2.
New Zealand Food Safety Authority	Not stated	• Has had the DAR reviewed by the Institute of Environmental Science and Research Limited (ESR). As a result, queries whether any assessment for presence of residual CTP2 targeting peptide was undertaken. • Believes comment required in the FAR on whether any assessment for residual targeting peptide was performed, and if not a justification for the assumption that the peptide was fully degraded should be provided.
Private (David MacClement)	2	• Intended to object to inclusion of GM material in foods for sale in NZ. • Having now read relevant parts of FSANZ's Assessment, believes the initial genetic modification was done with proper scientific care, and that the evaluation was done in accordance with the three primary objectives set out in section 18 of the FSANZ Act. • Consequently, supports option 2
Australian Food and Grocery Council	2	• Supports the application on the basis that FSANZ's assessment did not identify any risk to public health and safety • States companies and individuals can then made independent commercial decisions as to whether or not to use this product. • Believes GM labelling requirements will provide consumers with appropriate information on which to base informed choice.

Submitter	Option	Comments
NSW Food Authority	2	• Supports option 2 pending further consideration of the cost to government when enforcing GM food standards. • Believes the cost benefit analysis included in the DAR is insufficient, as enforcement costs for GM foods are higher than for other regulatory measures. • Intends to commence a process involving all jurisdictions to discuss this matter.
Queensland Health (on behalf of whole of Qld Govt)	2	• Supports option 2 on condition that the cost to government when enforcing GM food standards is addressed more fully in the FAR. • Considers the cost benefit analysis in the DAR is significantly lacking. • Detection of GM foods is more complex and expensive than other food regulatory measures and will impact on monitoring resources for Queensland. • Believes reliance on a paper trail for imported foods, to reduce reliance on lab testing, is of limited use. • Believes a national enforcement strategy for GM food, which includes education, needs to be progressed without further delay.
Private (Paul Elwell-Sutton)	1	• Opposes the application as FSANZ's current food-labelling regime is dominated by Australia and has denied the submitter the right to choose foods produced not using GM organisms. • Believes exemption from GM labelling for GM foods that are substantially equivalent to non-GM foods that are also free of novel DNA or proteins is an insult and denies him a basic human right. • Believes no foods derived from or using GM organisms should be allowed until a fully informative food labelling protocol is in place in New Zealand.

Attachment 4

Business Cost Calculator Report

Business Cost Calculator Report
A 592 - Food Derived From Glyphosate - Tolerant Soybean Mon 89788

Problem:	Before food derived from soybean line MON 89788 can enter the food supply in Australia and New Zealand, it must be assessed for safety and an amendment to the Code must be approved by the FSANZ board, and subsequently be notified to the Australia and New Zealand Food Regulation Ministerial Council. An amendment to the Code may only be gazetted, once the Ministerial Council process has been finalised.
Objective:	To determine whether it would be appropriate to amend the Code to approve the use of food derived from soybean line MON 89788 under Standard 1.5.2.

Policy Options

Option Name	Quickscan Result
Status Quo	FALSE
Approve food derived from soybean line MON 89788	FALSE

Compliance Cost Summary

Option Name:	Status Quo	
Businesses Affected:	N/A	
Type	**Cost per Business**	**Total Cost of Regulation**
N/A	N/A	N/A

Option Name:	Approve food derived from soybean line MON 89788	
Businesses Affected:	N/A	
Type	**Cost per Business**	**Total Cost of Regulation**
N/A	N/A	N/A

Caution should be used comparing options and interpreting results over time. The Business Cost Calculator does not estimate the future values of ongoing costs. Refer to the User Guidelines for further information.

This report contains summaries of compliance costs only. An assessment on the compliance cost in itself does not provide an answer to which policy option is the most effective and efficient one. Rather, it provides information which needs to be considered alongside other relevant factors and issues when deciding between alternative policy options.

Attachment 5

First Review Report

1. Introduction

On 11 February 2008, the Australia and New Zealand Food Regulation Ministerial Council (Ministerial Council) requested a First Review of Application A592, which seeks approval of food derived from a genetically modified (GM) soybean – namely, glyphosate-tolerant soybean line MON 89788. Approval of this Application involves a variation to Standard 1.5.2 – Food produced using Gene Technology, of the *Australia New Zealand Food Standards Code* (the Code).

Following a request for a review, FSANZ had three months to complete a response. In this instance, FSANZ was required to review the decision by 11 May 2008.

2. Objectives of Review

The objective of this Review is to reconsider the draft variation to Standard 1.5.2 in light of the Ministerial Council's grounds for review as outlined in Section 3 below.

3. Grounds for the Review requested by the Ministerial Council

A First Review of FSANZ's decision to approve Application A592 was sought on the grounds that the proposed amendment to Standard 1.5.2, to permit the sale and use of food derived from glyphosate-tolerant soybean line MON 89788, does not protect public health and safety.

The principal reason stated for this is that the Final Assessment Report for A592 does not address the issue of the persistence and uptake of foreign DNA in and across the gastrointestinal (GI) tract of mammals. In not addressing this issue in the Final Assessment Report, it is supposed that FSANZ has assumed one of the following:

1. that recombinant plant DNA is so completely degraded during digestion as to be effectively unavailable to facilitate perturbations along the GI tract or tissues and organs beyond it that could be of human health significance; or

2. that transfer of recombinant plant DNA to gut micro-organisms, gut epithelial and other cells, the blood stream and internal tissues and organs is so infrequent as to be unlikely to be of human health significance; or

3. that potential consequences of persistency and uptake of recombinant plant DNA in and across the GI tract are not likely to occur or not likely to be sufficiently different from persistence and uptake of naturally occurring DNA to warrant evaluation from a food safety perspective.

Numerous scientific publications are cited as evidence that, following ingestion of GM foods, foreign (recombinant) DNA can survive, to some degree, digestion in the GI tract where it can remain available for uptake by gut micro-organisms/gut cells or cross the intestinal mucosa into the bloodstream and be taken up by various tissues and cells where it may persist for some time. It is argued these publications challenge assumptions 1 and 2 above.

The Review Request acknowledges that persistence and uptake of DNA is not a phenomenon that is limited to recombinant-DNA and that the GI tract is exposed to a large amount of foreign DNA from non-GM sources. In addition, many obstacles exist in the transfer and uptake pathways that would limit the potential for such foreign DNA being functionally maintained and expressed.

However, a number of scientific articles are cited as evidence that foreign DNA will not always be rendered non-functional, and it is further claimed that the state of scientific knowledge is such that it is not yet possible to determine the consequences of this for human health. It is argued that confidence can therefore not be maintained in relation to assumption 3 above.

FSANZ is therefore requested to confirm or articulate clearly the rationale it uses for excluding such issues from consideration in the safety assessment.

4. Background

An Application was received from Monsanto Australia Ltd on 19 October 2006 to amend the Code to approve food derived from glyphosate-tolerant soybean line MON 89788. Standard 1.5.2 requires that GM foods undergo a pre-market safety assessment before they may be sold in Australia and New Zealand.

Soybean line MON 89788 has been genetically modified to be tolerant to the herbicide glyphosate. The glyphosate-tolerance trait is conferred by expression of the *cp4 epsps* gene derived from *Agrobacterium* sp. strain CP4. The *cp4 epsps* gene codes for an enzyme, 5-enolpyruvyl-3-shikimate phosphate synthase (EPSPS). The EPSPS enzyme is present in all plants, bacteria and fungi and is essential for aromatic amino acid biosynthesis. The normal mode of action of glyphosate is to inhibit the endogenous plant EPSPS, thus blocking the synthesis of aromatic amino acids in cells which subsequently leads to the death of the plant. In contrast to the plant EPSPS, the bacterial EPSPS is able to function in the presence of glyphosate, therefore expression of CP4 EPSPS in the plant allows continued production of aromatic amino acids in the presence of the herbicide.

FSANZ undertook a pre-market safety assessment of food derived from glyphosate-tolerant soybean line MON 89788 according to the safety assessment guidelines applied to all GM foods. The safety assessment included a full molecular characterisation of the genetic modification, an evaluation of the safety of the newly expressed CP4 EPSPS protein, and a comprehensive compositional analysis of the food. The conclusion of the safety assessment was that, on the basis of all the available evidence, food derived from soybean line MON 89788 is as safe as food derived from other soybean varieties.

5. Conclusions from the Final Assessment Report

The Executive Summary and Reasons for Decision, which were approved by the FSANZ Board in November 2007, are in this Report at **Attachment 2**.

The Board agreed to the recommendation at Final Assessment to approve food from glyphosate-tolerant soybean line MON 89788 in view of the findings of the safety assessment report that food derived from line MON 89788 is as safe as food derived from other soybean varieties.

6. Issues addressed in First Review

The issue of persistence and uptake of recombinant DNA, when ingested, is not unique or specific to Application A592, but rather is a general issue that has been the subject of extensive consideration and publication over the last 15 plus years. The issue was first addressed at the international level in 1991 by a joint FAO/WHO expert consultation which looked at strategies for assessing the safety of foods produced by biotechnology (WHO 1991). It was concluded that as DNA from all living organisms is structurally similar, the presence of recombinant DNA in food products, in itself, poses no additional health risk to consumers. Similar conclusions have been reached by other expert consultations and intergovernmental bodies which have been convened specifically to address the issue of the presence of antibiotic resistance genes in foods (WHO 1993, Karenlampi 1996). The safety of recombinant-DNA in foods has also been considered in a number of comprehensive literature reviews, where it has also been concluded that the scientific information available to date does not indicate any safety concerns (Jonas et al 2001, Gaye & Gillespie 2005, Flachowsky et al 2007, EFSA 2007).

FSANZ routinely monitors the scientific literature for studies relevant to the safety assessment of GM foods and is fully cognisant of the literature relating to the uptake and persistence of recombinant DNA when ingested as part of GM food. While the issue continues to be an active area of research and publication, FSANZ does not regard this as an issue that requires specific and explicit consideration for each and every application for GM food for the following reasons:

6.1 Recombinant DNA is no different to DNA from non-GM sources

All DNA is made up of the same chemical elements; recombinant DNA and DNA from non-GM sources is therefore composed of the same four nucleotides. Genetic modification results in the reassortment of sequences of nucleotides but leaves chemical structure unchanged. Recombinant DNA is therefore chemically identical to non-recombinant DNA. There is also very little that is unique about the sequences of recombinant DNA, as most gene constructs that are used for transformation are derived from naturally occurring gene sequences, the vast majority of which would have been encountered before in food, either because they are derived from plant genes, or from bacteria or plant viruses that are often found associated with food (e.g. *Bacillus subtilis*, a common soil bacterium from which *Bt* genes are derived, might often be found on the surface of fresh fruit and vegetables; the cauliflower mosaic virus from which promoter sequences are often derived is frequently present in fresh vegetables).

6.2 Human beings are exposed to large quantities of foreign DNA and other nucleic acids (e.g. RNA) from a wide variety of sources on a daily basis as part of the diet

Nucleic acids are a natural component of food. Their total amount varies according to the type of food. For example, edible offal and animal muscle tissue comprise a high content of both DNA and RNA (per gram of tissue), whereas plant storage tissues, such as grains or potatoes, contain less DNA and RNA because they contain less cell nuclei (Jonas et al 2001). Dietary intake of nucleic acid is therefore influenced heavily by the diet of individuals and varies widely, but has been estimated to be in the range 0.1-1.0 g/person/day (Doerfler & Schubbert 1997).

6.3 The presence of recombinant DNA in food does not increase the overall dietary intake of DNA

Genetic modification typically results in the introduction of one or two new genes into an organism's genome. Given the large size of plant genomes, the contribution made by recombinant DNA to the total DNA in the genome will be very small. For example, for corn, which has an average genome size of 2,292 Mb, transformed with an insert of approximately 5 kb, the inserted recombinant DNA will make up only 0.00022% of the total DNA in the genome (Jonas et al 2001).

6.4 Nucleic acids are broken down during food processing

Food processing may lead to partial or complete degradation or removal of DNA. Physical and chemical factors, such as shear forces, heat or pH, may cause random cleavage of DNA strands, thus reducing the average DNA length but not total DNA content (Jonas et al 2001). Some processes such as the purification of sugar and the production of refined oils will remove most, if not all, DNA.

A number of studies focussing on various thermal treatments applied to food during processing (e.g. canning, fermentation), indicate that most DNA (including recombinant DNA) will be reduced to lengths of approximately 300 base pairs or less (Ebbehoj & Thomsen 1991, Hupfer et al 1998, Straub et al 1999). DNA fragments of such size are unlikely to encode functional genes, since this would require not only the full coding region to be present but also the appropriate regulatory sequences (e.g. promoter, terminator).

6.5 Ingested nucleic acids are extensively broken down in the digestive tract

Irrespective of whether GM foods are subject to processing prior to consumption, nucleic acid will also be broken down during digestion. Ingested DNA is cleaved through acid hydrolysis and enzymatic digestion (especially by pancreatic and intestinal nucleases) into small DNA fragments and mixtures of mono-, di-, tri-, oligo- and polynucleotides, which are then further catabolised into sugar phosphates and purine and pyrimidine bases (Carver & Walker 1995).

The fate of ingested DNA has been extensively studied and is discussed in a number of reviews (e.g. Beever & Kemp 2000, Jonas et al 2001). Given the chemical and structural similarity of all DNA, there is no basis for considering that in vivo hydrolysis and absorption of recombinant DNA will be different from non-recombinant DNA.

While the vast majority of ingested DNA will be degraded in the GI tract, a number of studies, including one in humans, have demonstrated that this process may not completely degrade all ingested DNA, with some incompletely digested DNA fragments being absorbed and detected transiently in cells of the GI tract as well as blood, liver, spleen and other organs and tissues. The most quoted of these is the human study reported by Netherwood et al (2004) as well as the series of studies in mice reported by Schubbert et al (1994, 1997, and 1998).

In the Netherwood et al study, nineteen human volunteers (twelve with intact digestive tracts, seven with ileostomies[6]) were fed GM soy containing the *epsps* gene. The amount of recombinant DNA that survived passage through the small bowel varied between the seven ileostomists, with a maximum of 3.7% recovered from the stoma of one individual. This rate of recovery was similar to an endogenous soy gene, suggesting the recombinant DNA was digested similarly to other plant DNA. The *epsps* gene could not be detected in faeces from subjects with intact digestive tracts, suggesting that any DNA surviving digestion in the upper GI tract is readily degraded in the large intestine. The study also found evidence of pre-existing transfer of a fragment of the *epsps* gene between GM soy and a small number of micro-organisms in the small intestine of the ileostomists. The authors speculated this had occurred prior to commencement of the study. There was no evidence of the intact *epsps* gene being transferred. In subjects with intact digestive tracts, none of the endogenous bacteria in the faeces were found to contain any *epsps* gene fragments from GM soy.

In the studies reported by Schubbert et al, M13 bacteriophage DNA was fed to mice at high doses and transiently detected as fragments in various tissues including foetal tissue. The vast majority of cells identified as containing M13 DNA fragments appeared to be macrophages or other differentiated phagocytes of the immune system. The purpose of such cells is to destroy foreign macromolecules. It has been suggested that the relatively high frequency of cells that contained M13 DNA is probably related to the occurrence of unmethylated CpG sequences, which would stimulate macrophages and other immune cells to phagocytose the fragments (Beever and Kemp, 2000). Unmethylated CpG sequences are characteristic of bacterial DNA but not DNA in either plants or animals, therefore M13 DNA is probably not a good model for plant-derived recombinant DNA.

Other studies undertaken with livestock species ingesting GM plants (e.g. Einspanier et al 2001, Aulrich et al 2002, Reuter & Aulrich 2003, Tony et al 2003, Flachowsky et al 2005, Broll et al 2005, Mazza et al 2005) have confirmed that plant DNA may be readily detected in the tissues of animals. In some of these studies, small fragments of recombinant DNA were also detected in the GI tract or specifically the stomach, and in one case in the blood, liver, spleen and kidney (Mazza et al 2005), but so far, intact genes of recombinant-DNA origin have not been detected.

These results clearly indicate that the systemic uptake of ingested foreign DNA is a normal physiological process, and the demonstration of fragments of DNA in phagocytic cells should be expected as a natural consequence of that uptake. These cells provide immune surveillance of the digestive tract and other tissues, and recirculate frequently to the liver as a normal mechanism of removing debris. The rare appearance of foreign DNA fragments in a few foetal or neonatal cells should likewise not be of concern as it indicates that a few macromolecules have crossed the placenta and been engulfed by phagocytes of the foetus.

[6] An ileostomy involves resection of the terminal ileum and diversion of digesta via a stoma to a colostomy bag.

It should also come as no surprise that, with the improved sensitivity of analytical techniques, small fragments of recombinant DNA will occasionally be detected. The less frequent detection of recombinant DNA fragments probably reflects that recombinant DNA makes up only a very small proportion of the total DNA ingested (see 6.3 above).

6.6 Uptake and expression of foreign DNA by micro-organisms inhabiting the digestive tract is likely to be an extremely rare event

The horizontal DNA transfer of recombinant DNA into gut micro-organisms has been the subject of intense scientific scrutiny and debate, particularly in relation to the use of antibiotic resistance genes, and the possibility that such transfer could compromise the therapeutic use of antibiotics. Some studies are available which demonstrate that, in certain circumstances, foreign DNA may be taken up and expressed by micro-organisms, at least in vitro (e.g. Mercer et al 1999). To date, there is no evidence of transfer to and expression of recombinant DNA in bacteria under natural conditions. Transfer and expression has only been observed under laboratory conditions and only if homologous recombination is possible (Nielsen et al 1998). While such studies provide evidence of the possibility of DNA uptake by bacteria, they do not provide evidence that recombinant DNA poses any greater risk. The overwhelming scientific consensus is that, while theoretically possible, the likelihood of transfer and functional integration of recombinant DNA in gut micro-organisms is extremely low.

The gene transfer mechanisms by which bacteria may acquire new genes (conjugation, transduction and transformation) are well described and a number of comprehensive reviews on these processes are available (e.g. Levy & Miller 1989). In food, transfer by all three mechanisms is believed to be possible, at least from micro-organisms consumed in food, although studies on gene transfer in the human and animal gut are limited (Jonas et al 2001). The gut and the colon in particular are considered to be a favourable environment for such transfer because of the high density of micro-organisms; direct cell to cell contact favours conjugation, and natural transformation is also favoured because of the relatively high DNA concentration at the recipient cell surface (Paul 1992).

For free DNA however there is only a very low probability per gene and per passage through the GI tract, of uptake and stable integration into the genome of a bacterial cell. There are several reasons for this, which are extensively elaborated in Jonas et al (2001), but briefly:

- degradation of DNA through the gastric and ileal passage makes it highly unlikely that linear DNA molecules of sufficient size will enter the colon;
- for transformation by linear DNA the bacterial cell must be competent:

    - a bacteria is said to be competent if it is able to naturally take up DNA from the environment. Competence usually occurs at a particular stage in the bacterial growth cycle when the bacterium produces a protein called a competence factor. Only between 1-2% of microbial species are thought to be naturally competent;

- DNA transferred through transduction or transformation may be susceptible to restriction by bacterial restriction endonucleases, which cleave double-stranded DNA;
- in the case of linear DNA, homology with sequences in the bacterial genome is necessary for integration to occur;

- to be expressed, the transferred DNA must contain an intact coding region and be associated with the appropriate bacterial expression signals:

    - most recombinant DNA derived from GM plants will be linked to plant-specific expression signals which are unlikely to function in bacterial cells;

- To be maintained by the bacterial population, acquired DNA must confer a competitive advantage to the transformed cell.

Therefore, although bacteria possess sophisticated systems for DNA uptake from their environment, horizontal transfer into and expression of free recombinant DNA present in food is predicted to be an extremely rare event.

Given the similarity between recombinant DNA and non-recombinant DNA, both in terms of chemical structure as well as sequence, the likelihood of transfer and functional integration of recombinant DNA by gut micro-organisms will be theoretically the same as for non-recombinant DNA present in food. It might also be argued that, as recombinant DNA would represent only a very small proportion of the total DNA ingested in food, successful transfer of recombinant DNA to gut micro-organisms would be far less likely to occur than transfer of non-recombinant DNA.

6.7 Should a small proportion of ingested DNA survive digestion in the GI tract, mammals possess effective mechanisms to avoid incorporation of foreign DNA into the genome

Mammalian cells have evolved with several mechanisms of defence against the uptake, integration and continued expression of foreign DNA (Doerfler 1991). In addition to the initial degradation and/or excretion of foreign DNA that occurs following ingestion and the action of cells of the immune system e.g. phagocytes, to remove foreign macromolecules, most mammalian cells produce at least one DNase with exonuclease activity, and these would be expected to degrade most exogenous DNA, should it actually survive and be taken up by the cell (Jonas et al 2001). The nuclear membrane is also a strong barrier against the penetration of nucleic acids. Entry is tightly regulated by nuclear pores, with nuclear targeting signals required for penetration, especially in the case of cells that have finished their division and the nuclear envelope is not disrupted (Gorlick & Mattaj 1996, Guralnick et al 1996, Collas & Aelstrom 1997, Palacios et al 1997, Popov et al 1998, Zeimienovicz et al 1999, Saphire et al 2000). Should DNA succeed in penetrating the nucleus, and become integrated in the genome, the evidence indicates that any integrated foreign DNA is likely to be rendered inactive through targeted methylation (Doerfler 1991, Doerfler et al 1995, Orend et al 1995).

6.8 The risk posed by the presence of recombinant DNA in food is no different to that posed by non-recombinant DNA.

While the Review Request raises a number of interesting questions in relation to the potential impact on human health, should foreign DNA not be inactivated if taken up by cells, the studies cited (e.g. Palka-Santini et al 2003, Woodhams et al 2007, Rosenberg et al 2007) do not provide any compelling arguments that such health impacts, should they occur, are likely to be any greater with recombinant DNA compared to non-recombinant DNA.

The study by Malatesta et al (2002) on the ultrastructure of hepatocytes from mice fed GM soybean[7], is interesting in that the authors report that the GM soy-fed mice exhibited some slight but statistically significant ultrastructural differences in hepatocyte nuclei[8] relative to controls. Cells bearing slightly more irregularly shaped nuclei were postulated to be indicative of an increased metabolic rate and the slight increase in the number of nuclear pores was apparently suggestive of increased molecular trafficking between the nucleus and cytoplasm.

The study itself is quite unusual because it undertakes an investigation at the ultrastructural level in the absence of any clear evidence of effects in the liver at either the macroscopic or light microscopic level. Typically, ultrastructural investigations are only undertaken to identify an underlying mechanism if there is clear evidence of cellular change or clinical signs. In the Malatesta et al study only 100 cells/mouse were examined. Consequently the relevance of the subtle ultrastructural morphometrical changes observed are difficult to interpret, especially in the absence of any corroborating evidence of atypical liver activity (e.g. classical markers of liver cell damage). In addition, it is not clear that such effects, were they to be reproduced, would necessarily be attributable to the presence of recombinant DNA itself. The relevance of this study to the issue of persistence and uptake of recombinant DNA is therefore questionable.

The main objective of a GM food safety assessment is to identify whether new or altered hazards are present in the food as a result of the genetic modification, and if present to determine what risk, if any, they may pose to human health (Codex 2004, FSANZ 2007). Therefore, the key issue for FSANZ is whether the occurrence of recombinant-DNA in food poses any greater risk to human health, than that posed by the significantly larger amount of non-recombinant DNA already present in food.

In general, FSANZ considers the risk to be equivalent between recombinant and non-recombinant DNA and therefore does not regard this as an issue that requires explicit consideration for each and every GM food application. Rather, this issue need only be addressed if the molecular characterisation identifies an element or elements in the gene construct that may significantly increase the likelihood of recombinant DNA in GM food being taken up and stably incorporated in either gut micro-organisms or human cells. The constructs typically used to date contain coding and regulatory sequences that have been used many times before and are well known not to increase the likelihood of such events occurring.

In the case of MON 89788 soybean, the transferred DNA sequences consist of the following:

- the right and left border sequences from the *Agrobacterium* Ti plasmid;
- a chimeric promoter consisting of sequences derived from the 35S promoter from the figwort mosaic virus and an endogenous plant promoter from *Arabidopsis thaliana*;
- a chloroplast transit peptide sequence from *A. thaliana*;
- the *epsps* gene from *Agrobacterium* sp. strain CP4;
- the 3' untranslated sequence from the ribulose-1,5-bisphosphate carboylase small subunit *E9* gene from pea (*Pisum sativum*)

[7] The GM soy line used was glyphosate tolerant soybean line 40-3-2, not MON 89788.
[8] Irregularly shaped nuclei and increased numbers of nuclear pores.

With the exception of the right and left border sequences from the *Agrobacterium* Ti plasmid, none of the other components of the MON 89788 gene construct contain sequences or elements that might conceivably increase the likelihood of transfer to and integration into the genome of either bacterial or human cells. The purpose of the right and left border sequences is to facilitate transfer of foreign DNA to the plant genome. Presence of the border sequences alone however is not sufficient to mediate transfer; transfer also requires the action of virulence factors (proteins) which are supplied in trans during the plant transformation process.

In the absence of these virulence factors, which would be the case for ingested GM food, the presence of the right and left border sequences would have no such facilitating effect.

6.9 Conclusion

The transferred DNA in MON 89788 does not contain any genetic elements which may significantly increase the likelihood of recombinant DNA in GM food being taken up and stably incorporated into the genome of either gut micro-organisms or human cells. Given this, FSANZ does not consider that the issue of persistence and uptake of recombinant DNA requires specific consideration in the safety assessment of food derived from glyphosate-tolerant soybean line MON 89788; consideration of such issues is already implicit in the molecular characterisation component of the safety assessment.

7. Review Options

There are three options proposed for consideration under this Review:

1. re-affirm approval of the draft variation to Standard 1.5.2 as notified to the Council; or

2. re-affirm approval of the draft variation to Standard 1.5.2, subject to any amendments FSANZ considers necessary; or

3. withdraw approval of the draft variation to Standard 1.5.2 of the Code as notified to the Council.

8. Decision

FSANZ has considered the issues raised by the Ministerial Council in relation to Application A592 – Food derived from glyphosate-tolerant soybean line 89788.

The First Review concludes that the preferred review option is Option 1. FSANZ has decided to re-affirm the variation to Standard 1.5.2 of the Code to permit the sale of food derived from glyphosate-tolerant soybean line 89788, as detailed in **Attachment 1**.

The recommended option is Option 1.

Decision

FSANZ re-affirms the variation to Standard 1.5.2 to permit the sale of food derived from glyphosate-tolerant soybean line 89788.

9. Implementation and review

The draft variation to Standard 1.5.2 of the Code will come into effect on the date of gazettal.

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Attachments

1. Draft variation to the *Australia New Zealand Food Standards Code.*
2. Executive Summary and Statement of Reasons from the Final Assessment Report

Attachment 1

Draft variation to the *Australia New Zealand Food Standards Code*

Standards or variations to standards are considered to be legislative instruments for the purposes of the Legislative Instruments Act (2003) and are not subject to disallowance or sunsetting.

To commence: on gazettal

[1] ***Standard 1.5.2*** *of the Australia New Zealand Food Standards Code is varied by inserting in the* Table to clause 2 –

Food derived from glyphosate-tolerant soybean line MON 89788	

Attachment 2

Executive Summary and Reasons for Decision from the Final Assessment Report

An Application has been received from Monsanto Australia Limited to amend the *Australia New Zealand Food Standards Code* (the Code) to approve food derived from genetically modified (GM) herbicide-tolerant soybean MON 89788. Standard 1.5.2 – Food produced using Gene Technology requires that GM foods undergo a pre-market safety assessment before they may be sold in Australia and New Zealand.

Soybean MON 89788 has been genetically modified to be tolerant to the herbicide glyphosate. FSANZ has undertaken a safety assessment of glyphosate-tolerant soybean MON 89788. If approved, food derived from glyphosate-tolerant soybean MON 89788 may enter Australia and New Zealand as imported products. It is not intended that MON 89788 be cultivated in Australia or New Zealand

The herbicide tolerance trait introduced into glyphosate-tolerant soybean MON 89788 is conferred by expression in the plant of an enzyme, CP4 EPSPS, derived from a common soil bacterium. No marker genes are present in glyphosate-tolerant soybean MON 89788.

Safety assessment

FSANZ has completed a comprehensive safety assessment of food derived from glyphosate-tolerant soybean MON 89788, as required under Standard 1.5.2. The assessment included consideration of (i) the genetic modification to the plant; (ii) the potential toxicity and allergenicity of the novel protein; and (iii) the composition of glyphosate-tolerant soybean MON 89788 compared with that of conventional soybean.

The assessment of this Application identified no public health and safety concerns. On the basis of the available evidence, including detailed studies provided by the Applicant, food derived from glyphosate-tolerant MON 89788 is considered as safe and wholesome as food derived from commercial soybean varieties.

Labelling

Foods derived from glyphosate-tolerant soybean MON 89788 will be required to be labelled as genetically modified if novel DNA and/or novel protein is present in the final food. Studies conducted by the Applicant show that the novel protein is present in the unprocessed grain. Highly refined products, such as soybean oil, will not require labelling if they do not contain novel protein or DNA.

Labelling addresses the requirement of paragraph 18(1)(b) of the *Food Standards Australia New Zealand Act 1991*; provision of adequate information relating to food to enable consumers to make informed choices.

Impact of regulatory options

Two regulatory options were considered in the assessment: (1) no approval; or (2) approval of food derived from glyphosate-tolerant soybean MON 89788 based on the conclusions of the safety assessment.

Following analysis of the potential costs and benefits of each option on affected parties (consumers, the food industry and government), approval of this application is the preferred option as the potential benefits to all sectors outweigh the costs associated with the approval.

Purpose

The Applicant seeks amendment to Standard 1.5.2 – Food produced using Gene Technology, to include food derived from glyphosate-tolerant soybean MON 89788 in the Table to clause 2.

Decision

Amend Standard 1.5.2 – Food produced using Gene Technology, to include food derived from glyphosate-tolerant soybean MON 89788 in the Table to clause 2.

Reasons for Decision

An amendment to the Code approving food derived from glyphosate-tolerant soybean MON 89788 in Australia and New Zealand is approved on the basis of the available scientific evidence, for the following reasons:

- the safety assessment did not identify any public health and safety concerns associated with the genetic modification used to produce glyphosate-tolerant soybean MON 89788;
- food derived from glyphosate-tolerant soybean MON 89788 is equivalent to food from other commercially available soybean varieties in terms of its safety for human consumption and nutritional adequacy;
- labelling of certain food fractions derived from glyphosate-tolerant soybean MON 89788 will be required if novel DNA and/or protein is present in the final food; and
- a regulation impact assessment process has been undertaken that also fulfils the requirement in New Zealand for an assessment of compliance costs. The assessment concluded that the most appropriate option is option 2, an amendment to the Code.

Consultation

The Initial Assessment was advertised for public comment between 13 December 2006 and 7 February 2007. A total of six submissions were received during this period. The Draft Assessment was advertised for public comment between 8 August 2007 and 19 September 2007. A total of nine submissions were received. A summary of these is provided in **Attachment 3** to this Report.