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7 December 2005

DRAFT ASSESSMENT REPORT

APPLICATION A548

FOOD FROM CORN ROOTWORM-PROTECTED & GLYPHOSATE-TOLERANT CORN MON 88017

DEADLINE FOR PUBLIC SUBMISSIONS: 6pm (Canberra time) 1 February 2006
SUBMISSIONS RECEIVED AFTER THIS DEADLINE
WILL NOT BE CONSIDERED

(See 'Invitation for Public Submissions' for details)

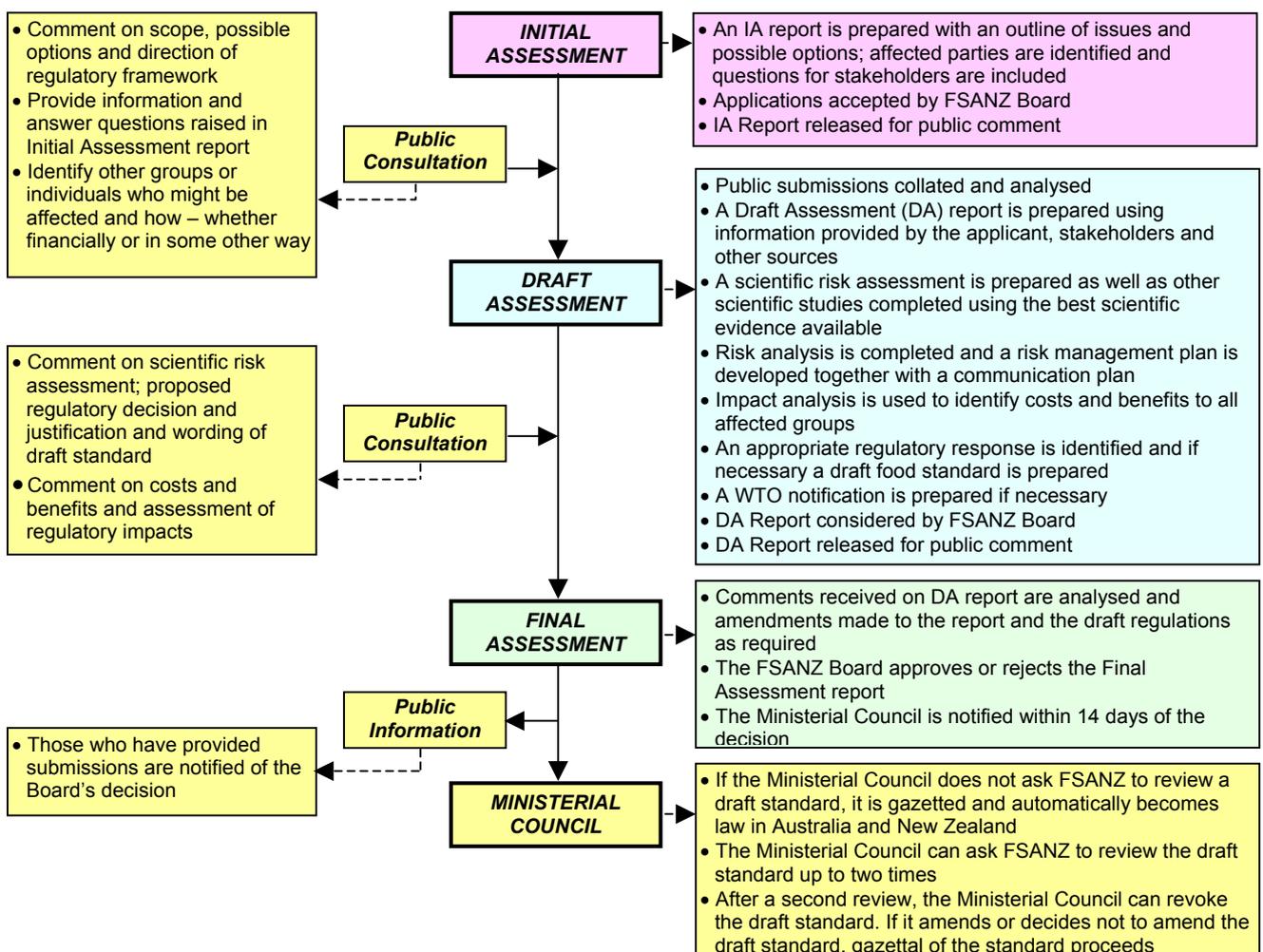
FOOD STANDARDS AUSTRALIA NEW ZEALAND (FSANZ)

FSANZ's role is to protect the health and safety of people in Australia and New Zealand through the maintenance of a safe food supply. FSANZ is a partnership between ten Governments: the Australian Government; Australian States and Territories; and New Zealand. It is a statutory authority under Commonwealth law and is an independent, expert body.

FSANZ is responsible for developing, varying and reviewing standards and for developing codes of conduct with industry for food available in Australia and New Zealand covering labelling, composition and contaminants. In Australia, FSANZ also develops food standards for food safety, maximum residue limits, primary production and processing and a range of other functions including the coordination of national food surveillance and recall systems, conducting research and assessing policies about imported food.

The FSANZ Board approves new standards or variations to food standards in accordance with policy guidelines set by the Australia and New Zealand Food Regulation Ministerial Council (Ministerial Council) made up of Australian Government, State and Territory and New Zealand Health Ministers as lead Ministers, with representation from other portfolios. Approved standards are then notified to the Ministerial Council. The Ministerial Council may then request that FSANZ review a proposed or existing standard. If the Ministerial Council does not request that FSANZ review the draft standard, or amends a draft standard, the standard is adopted by reference under the food laws of the Australian Government, States, Territories and New Zealand. The Ministerial Council can, independently of a notification from FSANZ, request that FSANZ review a standard.

The process for amending the *Australia New Zealand Food Standards Code* is prescribed in the *Food Standards Australia New Zealand Act 1991* (FSANZ Act). The diagram below represents the different stages in the process including when periods of public consultation occur. This process varies for matters that are urgent or minor in significance or complexity.



INVITATION FOR PUBLIC SUBMISSIONS

FSANZ has prepared a Draft Assessment Report of Application A548 and prepared a draft variation to the *Australia New Zealand Food Standards Code* (the Code).

FSANZ invites public comment on this Draft Assessment Report based on regulation impact principles and the draft variation to the Code for the purpose of preparing an amendment to the Code for approval by the FSANZ Board.

Written submissions are invited from interested individuals and organisations to assist FSANZ in preparing the Final Assessment for this Application. Submissions should, where possible, address the objectives of FSANZ as set out in section 10 of the FSANZ Act. Information providing details of potential costs and benefits of the proposed change to the Code from stakeholders is highly desirable. Claims made in submissions should be supported wherever possible by referencing or including relevant studies, research findings, trials, surveys etc. Technical information should be in sufficient detail to allow independent scientific assessment.

The processes of FSANZ are open to public scrutiny, and any submissions received will ordinarily be placed on the public register of FSANZ and made available for inspection. If you wish any information contained in a submission to remain confidential to FSANZ, you should clearly identify the sensitive information and provide justification for treating it as commercial-in-confidence. Section 39 of the FSANZ Act requires FSANZ to treat in-confidence, trade secrets relating to food and any other information relating to food, the commercial value of which would be, or could reasonably be expected to be, destroyed or diminished by disclosure.

Submissions must be made in writing and should clearly be marked with the word 'Submission' and quote the correct project number and name. Submissions may be sent to one of the following addresses:

**Food Standards Australia New Zealand
PO Box 7186
Canberra BC ACT 2610
AUSTRALIA
Tel (02) 6271 2222
www.foodstandards.gov.au**

**Food Standards Australia New Zealand
PO Box 10559
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NEW ZEALAND
Tel (04) 473 9942
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Submissions need to be received by FSANZ by 6pm (Canberra time) 1 February 2006.

Submissions received after this date will not be considered, unless agreement for an extension has been given prior to this closing date. Agreement to an extension of time will only be given if extraordinary circumstances warrant an extension to the submission period. Any agreed extension will be notified on the FSANZ Website and will apply to all submitters.

While FSANZ accepts submissions in hard copy to our offices, it is more convenient and quicker to receive submissions electronically through the FSANZ website using the Standards Development tab and then through Documents for Public Comment. Questions relating to making submissions or the application process can be directed to the Standards Management Officer at the above address or by emailing slo@foodstandards.gov.au.

Assessment reports are available for viewing and downloading from the FSANZ website. Alternatively, requests for paper copies of reports or other general inquiries can be directed to FSANZ's Information Officer at either of the above addresses or by emailing info@foodstandards.gov.au.

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Executive Summary and Statement of Reasons

Monsanto Australia Limited is seeking approval in the *Australia New Zealand Food Standards Code* (the Code) for food derived from corn line 88017 (MON 88017), a variety that has been genetically modified (GM) for insect-protection and herbicide-tolerance. 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. Foods derived from corn line 88017 could be imported into Australia and New Zealand, however there are no intentions to cultivate corn line 88017 in either country.

The dual trait introduced into corn line 88017 involves the addition of two novel genes. One gene confers protection from corn rootworm, a significant insect pest of corn in the Northern hemisphere. Protection is conferred by the expression in the plant of a bacterially-derived insecticidal protein (from the family of *Bt-δ* endotoxins) that is specific for the rootworm. The second gene confers tolerance to the herbicide glyphosate by expression in the plant of an enzyme, CP4 EPSPS, also derived from a common soil bacterium. No marker genes are present in MON 88017 corn.

Safety assessment

FSANZ has completed a comprehensive safety assessment of food derived from corn line 88017, as required under Standard 1.5.2 in the Code. The assessment included consideration of (i) the genetic modification to the plant; (ii) the potential toxicity and allergenicity of the two novel proteins; (iii) the composition of MON 88017 corn grain compared with that of conventional corn grain; and (iv) the ability of MON 88017 corn grain to support typical growth and wellbeing in animals.

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 corn line 88017 is considered as safe and wholesome as food derived from other commercial corn varieties.

Labelling

Foods derived from corn line 88017 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 novel proteins are present in the corn grain in very low amounts, however where processing removes plant DNA and proteins, labelling would not be required.

Labelling addresses the requirement of section 10(1)(b) of the Act; 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 corn line 88017 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.

Consultation

The Initial Assessment was advertised for public comment between 15 December 2004 and 5 February 2005. A total of 139 submissions were received during this period and a summary of these is attached to this report. All but five of the submissions were from New Zealand. A significant proportion of these were brief letters submitted as part of a campaign of opposition to GM foods.

FSANZ has taken the submitters comments into account in preparing the draft assessment of this application. Specific issues relating to corn line 88017 have been addressed in the report.

Decision

Food derived from insect-protected and glyphosate-tolerant corn line 88017 is as safe for human consumption as food from other commercial corn varieties. FSANZ recommends that food derived from corn line 88017 be approved.

Statement of Reasons

An amendment to the Code approving food derived from corn line 88017 in Australia and New Zealand is recommended 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 corn line 88017;
- food derived from corn line 88017 is equivalent to food from other commercially available corn varieties in terms of its safety for human consumption and nutritional adequacy;
- labelling of certain food fractions derived from corn line 88017 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 amendment to the Code is of net benefit to both food producers and consumers.

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

Public submissions are now invited on this Draft Assessment Report.

1. Introduction

An Application was received from Monsanto Australia Limited on 5 October 2004 seeking approval for food derived from insect-protected, glyphosate-tolerant corn line 88017 (MON 88017) under Standard 1.5.2 – Food Produced Using Gene Technology, in the Code.

The genetic modification in MON 88017 is a dual trait introduced by the transfer of the following genes derived from bacterial sources:

- the synthetic *cry3Bb1* gene which is the coding sequence for a close variant of the Cry3Bb1 protein, derived from *Bacillus thuringiensis*, and one of the family of Cry3Bb proteins with specific insecticidal activity; and
- the *cp4 epsps* gene which is the coding sequence for the native CP4 EPSPS protein, derived from *Agrobacterium* sp. strain CP4, an enzyme that confers tolerance to the herbicide glyphosate.

A Draft Assessment of the Application has been completed, including a comprehensive safety evaluation, and public comment is now being sought prior to preparation of the Final Assessment of the Application.

2. Regulatory Problem

Standard 1.5.2 requires that a genetically modified (GM) food undergo a pre-market safety assessment before it 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.

The Applicant, Monsanto Australia Limited, has developed a new variety of insect-protected and herbicide-tolerant corn, referred to as MON 88017, primarily for agronomic purposes. Before food derived from this corn 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.

The Applicant therefore seeks amendment to Standard 1.5.2 to include food derived from corn line 88017 in the Table to clause 2.

3. Objective

In developing or varying a food standard, FSANZ is required by its legislation to meet three primary objectives, which are set out in section 10 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.

The key objectives of this assessment of MON 88017 corn are therefore the protection of public health and safety and the provision of adequate information to consumers. In fulfilling these objectives, FSANZ will also have regard for the need for standards to be based on a risk analysis using the best available scientific evidence, and the benefits of an efficient and internationally competitive food industry.

4. Background

In the northern hemisphere, corn plants are susceptible to damage from feeding by a Coleopteran insect pest, corn rootworm (*Diabrotica* spp.). The Applicant has previously developed two separate genetically modified (GM) inbred lines, one containing a trait that confers protection to the corn rootworm, and a second line that contains a trait conferring tolerance to the broad-spectrum herbicide glyphosate. These two inbred GM lines each containing the individual traits have previously been crossed using traditional plant breeding techniques to produce a corn hybrid with the dual trait. The traditional breeding process is considered inefficient, and requires long development times.

Corn line 88017 has therefore been developed using a two-gene insertion event that simultaneously creates corn rootworm-protection and glyphosate-tolerance in a single GM line. The purpose of the modification is to provide growers with access to a variety of elite corn germplasms containing both agronomic traits.

Corn line 88017 expresses a variant of the Cry3Bb1 protein isolated from the common soil bacterium *Bacillus thuringiensis* (*Bt*) subspecies *kumamotoensis*. This protein is toxic to specific insects, including three significant pests of corn: Western corn rootworm (*Diabrotica vigifera*), Northern corn rootworm (*Diabrotica berberis*) and Mexican corn rootworm (*Diabrotica vigifera zea*).

In addition, corn line 88017 expresses the enzyme 5-enolpyruvyl-3-shikimate phosphate synthase (EPSPS) from *Agrobacterium* sp. strain CP4. This enzyme, which is essential for aromatic amino acid synthesis, has a lower affinity for glyphosate compared to the plant version of EPSPS. The normal mode of action of the herbicide is to bind to the plant EPSPS, blocking the enzymic activity, which results in a lack of aromatic amino acids leading to the death of plant cells. As the bacterial form of the enzyme has a lower binding affinity for glyphosate, expression of CP4 EPSPS in the plant cells allows continued production of aromatic amino acids in the presence of the herbicide.

An insect-protected GM corn expressing the Cry3Bb1 protein only, known as event MON 863, has already been assessed by FSANZ and was approved under Standard 1.5.2 for use in Australia and New Zealand in 2003 (Application A484). MON 863 is also already approved in Japan and the United States and has recently been assessed as safe for human consumption by the European Food Safety Authority (EFSA, 2004).

4.1 Corn products

Corn, together with rice and wheat, is one of the most important cereal crops in the world with total production of 591 million tonnes in 2000 (FAOSTAT Database 2001). The majority of grain and forage derived from maize is used in animal feed. Maize grain is also used for industrial products such as ethyl alcohol and highly refined starch. Domestic production of corn in Australia and New Zealand is supplemented by the import of a small amount of corn-based food ingredients, largely high-fructose corn syrup, which is not currently manufactured in either Australia or New Zealand. Such products are processed into breakfast cereals, baking products, extruded confectionery and corn chips. Other food ingredients such as oils and cornstarch are also imported and used by the food industry for the manufacture of dessert mixes and canned foods.

4.2 Overseas regulatory status

Applications to permit the use of corn varieties containing event MON 88017 have been submitted to multiple authorities in the United States (USDA, USFDA, EPA). In 2001, the EPA established a time-limited exemption from the requirements of a tolerance for *Bt* Cry 3Bb1 proteins and the genetic material necessary for their production in all commodities (EPA, 2001,a). An application to the EPA to amend the exemption by removing the time limitation was made in 2003. The EPA previously has reviewed and established an exemption from the requirement of a tolerance for CP4 EPSPS and the genetic material necessary for the production of this protein in or on all raw agricultural commodities.

An environmental approval from the Japanese Ministry of Agriculture, Forestry and Fisheries (MAFF) for MON 88017 was obtained on April 23, 2003. The applicant states that regulatory submissions for import and production approvals will be made to countries that import US corn grain. These will include Japanese Ministry of Health, Labour and Welfare (MHLW) and MAFF (for use as animal feed), as well as the Canadian Food Inspection Agency (CFIA) and Health Canada. For countries that do not have a formal approval process, notifications of import will be made.

5. Relevant Issues

5.1 Safety of food from corn line 88017

Corn line 88017 has been assessed for safety according to the guidelines prepared by FSANZ¹. 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.

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

In conducting a safety assessment of food derived from MON 88017 corn, 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;
- (iv) potential for the newly expressed proteins to be either allergenic or toxic in humans; and
- (v) nutritional adequacy of MON 88017 corn in animal feeding studies.

5.1.1 History of Use

Corn (*Zea mays L*), otherwise known as maize, is the world's third leading cereal crop, behind wheat and rice, and is grown in over 25 countries worldwide. Corn-derived products are routinely used in a large number and diverse range of foods and have a long history of safe use. Products derived from corn line MON 88017 may include flour, breakfast cereals, oil, high fructose corn syrup, and other starch products.

5.1.2 Description of the Genetic Modification

MON 88017 corn was generated through the transfer of the *cp4 epsps* and *cry3Bb1* genes to an inbred hybrid corn line, A x Hi-II. 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) that continues to function in the biochemical pathway producing aromatic amino acids in a plant that has been sprayed with glyphosate, when the plant's own EPSPS enzyme has been inactivated by the herbicide. The *cry3Bb1* gene is derived from the soil bacterium *Bacillus thuringiensis* subspecies *kumamotoensis* and encodes the Cry3Bb1 protein, which is selectively toxic to certain Coleopteran insects in the larval stage including corn rootworm. The DNA sequence of the *cry3Bb1* gene transferred to MON 88017 is a close variant of the native gene sequence and has been designed to encode the Cry3Bb1 variant protein with enhanced insecticidal activity against corn rootworm. There was no transfer of bacterial antibiotic resistance marker genes in this modification.

Detailed molecular and genetic analyses of MON 88017 corn indicate that the transferred genes are stably integrated into the plant genome as single linked copies at the same insertion site, and are inherited in subsequent generations according to predicted patterns of inheritance.

5.1.3 Characterisation of Novel Protein

Two novel proteins are expressed in MON 88017 corn – CP4 EPSPS and the Cry3Bb1 variant protein. The mature CP4 EPSPS in MON 88017 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 Cry3Bb1 in MON 88017 differs from the native Cry3Bb1 by six amino acid changes, which were deliberately introduced to enhance insecticidal activity and facilitate laboratory manipulations. The Cry3Bb1 protein is also present in GM corn line MON 863, which was approved in 2003. The variant in MON 88017 differs from that in MON 863 by only one amino acid.

Both novel proteins are expressed at relatively low levels in MON 88017 plants. The mean level of CP4 EPSPS in grain was 5.8 µg/g dry weight, with concentrations in other plant tissues ranging from 390-57 µg/g dry weight. In general, the level of CP4 EPSPS protein declined during the growing season. The mean Cry3Bb1 protein level in the grain was 15 µg/g dry weight, with concentrations in other tissues ranging from 570-25 µg/g dry weight.

5.1.4 *Potential toxicity and allergenicity*

The two novel proteins present in MON 88017 corn have been assessed previously for safety; the CP4 EPSPS protein is present in approved lines of canola, cotton, soybean, potato and other corns. Previous assessments have shown that CP4 EPSPS administered directly to animals at a high dose is not toxic, and the evidence indicates that the protein shows no potential to be allergenic in humans. Given its widespread use in approved glyphosate-tolerant crops, it now has a history of safe use in food over 10 years.

In considering the potential toxicity and allergenicity of the Cry3Bb1 variant protein, it is worth noting that *Bt* formulations containing Cry3Bb1 have been used safely in agriculture since 1995. Two separate acute toxicity studies in mice using the individual Cry3Bb1 variant proteins present in MON 88017 and MON 863 respectively (sequences differ by one amino acid), confirmed the absence of mammalian toxicity in each case. Bioinformatic studies confirmed the absence of any significant amino acid similarity with known protein toxins and allergens, and *in vitro* digestibility studies demonstrated that Cry3Bb1 variants are rapidly degraded in the stomach following ingestion. Furthermore, processing involving heat treatment renders the Cry3Bb1 variant protein non-functional (i.e. unable to exert a toxic effect in insects). This weight of evidence indicates that the Cry3Bb1 variant protein is not toxic and is unlikely to pose an allergenic risk to humans.

5.1.5 *Comparative Analyses*

Compositional studies were conducted to establish the nutritional adequacy of MON 88017 corn compared to the non-GM control line and conventionally produced commercial corn varieties. The constituents measured were proximate analysis, fatty acids, amino acids, vitamins, minerals, secondary metabolites, and anti-nutrients such as phytic acid.

In general, no differences of biological significance were observed between MON 88017 corn and its non-GM counterpart. A reduction of approximately 23% in vitamin B₁ levels was observed in MON 88017 samples, however the levels were well within the literature range for this nutrient derived from conventionally bred corns. As vitamin B₁ levels in corn are generally low, a reduction of this magnitude is not considered to be nutritionally significant, especially as human food. Other minor differences in fatty acid or amino acid constituents were not indicative of an overall pattern of change that could be attributed to the modification. Grain from MON 88017 corn is therefore considered to be compositionally equivalent to grain from other commercial corn varieties.

5.1.6 *Nutritional Impact*

The detailed compositional studies are considered adequate to establish the nutritional adequacy of food derived from MON 88017 corn. Nevertheless, two animal feeding studies were considered as supplementary information – one in rats and one in catfish.

There were no observed differences in the general health and wellbeing of animals fed diets containing grain from MON 88017 corn compared to a standard diet.

5.1.7 Conclusion of the safety assessment

No potential public health and safety concerns have been identified in the comprehensive assessment of corn line MON 88017. On the basis of the data provided in this Application, and other available information, food derived from MON 88017 corn is considered as safe and wholesome as food derived from other corn varieties.

5.2 Labelling

Under Standard 1.5.2, GM food must be labelled if novel DNA or protein is present in the final food and also where the food has altered characteristics. Low amounts of CP4 EPSPS and Cry3Bb1 proteins were detected in corn grain and therefore some processed food products, such as cornflour, derived from MON 88017 grain would be required to be labelled as GM. However, labelling would not be required on more highly processed products that contain no novel DNA or protein. Such products include high fructose corn syrup and oil.

5.3 Issues arising from public submissions

5.3.1 Enforcement costs

The Queensland Environmental Health Unit considers that rising costs associated with the enforcement of the standard for GM foods must be addressed by demanding that future GM food approvals be contingent upon the applicants providing detection methodology that is freely available to all regulatory agencies to minimise cost increases associated with monitoring and to promote national consistency in enforcement of the standard. A national repository for suitable reference material, provided by each Applicant as a condition of approval, should also be established.

5.3.1.1 Response

Although detection methods for GM plant lines can be event-specific, certain analytical material is suitable for detecting a number of approved GM lines with the same introduced genetic trait, and routinely can distinguish a GM from a non-GM source when genetic material is present. For example, analytical methods and reagents necessary for detection of the *cp4 epsps* gene are common to the majority of approved glyphosate-tolerant plant lines. The usefulness of such material in detecting GM from non-GM varieties 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.

Labelling requirements under Standard 1.5.2 – Food Produced Using Gene Technology call for food manufacturers to seek and maintain documentation relating to the GM status of individual ingredients used in their products. In approving the expanded labelling requirements for GM foods in 2000, Health Ministers indicated that the purpose of the paper trail was to reduce the reliance on laboratory testing of foods as the sole tool for enforcement of the standard.

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. Australia's current system of food regulation provides for the discussion of such issues by the Implementation Sub Committee (ISC). 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.

5.3.2 *Cost/benefit analysis*

The South Australian Department of Health noted that

- (i) The RIS discussed the potential costs and benefits to growers of MON 88017 corn, despite the lack of intention to grow this corn in Australia;
- (ii) Approval for food derived from MON 88017 corn in Australia (and New Zealand) could increase the likelihood of the Applicant seeking permission for commercial release through the Office of the Gene Technology Regulator (OGTR); and
- (iii) The RIS appears to overstate the benefits to industry, as the new variety of corn does not offer any significantly different nutritional or processing qualities to give rise to new products.

5.3.2.1 Response

- (i) The RIS included the potential agricultural benefits to corn farmers from the use of MON88017 corn to reflect the possibility of lower primary production costs and higher yields, which would be likely to have a positive overall effect on the cost of producing corn food and feed products.
- (ii) Corn rootworm is a Northern hemisphere pest species and therefore MON 88017 corn is not specifically suitable for cultivation in Australia or New Zealand. In addition, corn is not an economically important agricultural crop in Australia and the majority of processed corn products used in food manufacture are imported.
- (iii) Corn farmers select the most appropriate cultivars for the prevailing agricultural conditions. Where there is demand for both introduced traits, the development of MON 88017 corn represents a reasonable improvement in production efficiency and would be likely to contribute to lower production costs. As corn is a global commodity and large amounts of co-mingled grain enter the supply chain, approval for MON 88017 corn would allow any potential cost savings to be passed to Australian food manufacturers who import processed corn for use in domestic food production. In turn, it is likely that the availability of less costly ingredients will be reflected in lower retail prices for products containing corn.

The agronomic traits introduced into MON 88017 corn do not offer consumers any direct nutritional benefits, and therefore do not give rise to new products with enhanced properties. Grain from MON 88017 corn would not be segregated from other commercially produced grain and would not attract a premium price.

5.3.3 *Safety of glyphosate and Bt proteins*

Physicians and Scientists for Responsible Genetics (PSRG) and others consider that the use of herbicide on glyphosate-tolerant crops such as MON 88017 corn poses an unacceptable risk to human health. In addition, PSRG claims that the safety to humans and animals of the Bt endotoxin is yet to be established. There is also a suggestion that a study reporting higher lignin content in various lines of GM corn (MON 810, Bt 11 and Bt 176) implies that pleiotropic effects in Bt-expressing corn can bring about compositional changes that could have adverse nutritional effects.

5.3.3.1 Response

Safety of glyphosate

It is normal agricultural practice to use a range of different herbicides on conventional crop plantings at appropriate stages of plant development. The use of a herbicide-tolerant crop generally results in an altered regimen for weed treatment in favour of the corresponding herbicide. In the case of GM glyphosate-tolerant crops, the modification allows the use of glyphosate at various stages of cultivation, whereas for traditional crops a range of herbicides are used at different times. Overall, the result of this altered spraying regimen is a greater reliance on one herbicide with a concomitant reduction in the use of other herbicides to treat weed infestations.

Because of its low toxicity to humans and to the environment, glyphosate is used widely in agriculture. As reflected in the Code in Standard 1.4.2 Maximum Residue Limits (MRL, Australia only), its use is permitted in the production of a broad range of human foods including a variety of fruits, vegetables, nuts and cereal grains. The purpose of the MRL is to set a level of residue that does not adversely affect human health while allowing good agricultural practice. The MRL applies to food, irrespective of whether it has been produced from a commercial non-GM or GM crop.

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

It is important to note that GM techniques are not the only means of obtaining herbicide-tolerant crop plants. These occasionally occur naturally, or can be enhanced by conventional plant breeding. The MRL for a herbicide such as glyphosate applies to all relevant foods whether produced from GM or non-GM sources, and thus GM varieties have to meet identical food residue standards.

Safety of Bt proteins

The use of *Bt* as a microbial pest control agent has been comprehensively evaluated under the International Programme on Chemical Safety (IPCS), a cooperative programme of United Nations Environment Programme (UNEP), International Labour Organisation (ILO) and the World Health Organisation (WHO). An Environmental Health Criteria (EHC) monograph on *Bacillus thuringiensis* was published in 1999², following an expert review and evaluation of the risks to human health and the environment from exposure to *Bt* products.

The review noted that the bacterium is a key source of genes for providing pest resistance in plants and microorganisms as pest control agents in GM organisms. Bt products account for approximately 90% of the world market in microbial pest control agents, and are used by the organic farming industry. Commercial Bt formulations have been used as bioinsecticides for foliage, soil, water environments or food storage facilities. In summarising the findings of the evaluation on exposure and effects on humans, the IPCS report states:

The field application of Bt products can result in considerable aerosol and dermal exposure of workers. Agricultural uses of Bt can result in Bt contamination of potable water and food. With the exception of case reports on ocular and dermal irritation, no adverse health effects have been documented after occupational exposure to Bt products.

Bt has not been reported to cause adverse effects on human health when present in drinking-water or food.

The Organisation for Economic Cooperation and Development (OECD) Task Force for the Safety of Novel Foods and Feeds has produced a series of consensus documents that describe the key compositional considerations for new varieties of food commodities such as corn, canola, soybean, sugar beet and potato. The consensus document for corn³ describes the parameters that developers of new corn varieties should measure. Critical components include key nutrients (those components with a substantial impact in the overall diet), and key toxicants (toxicologically significant compounds known to be inherently present). This approach also includes analyses of secondary plant metabolites as further indicators of the absence of unintended effects of the genetic modification on the metabolism of the plant.

FSANZ considers that the use of the consensus document for corn in the assessment of MON 88017 corn provides assurance that key compositional parameters, critical to the safety and nutritional quality of the food for humans, have been analysed and compared to an appropriate (isogenic) comparator and to a spectrum of reference plant lines with an accepted safe history of use.

² Environmental Health Criteria 217, Microbial Pest Control Agent *Bacillus thuringiensis*, WHO, Geneva, 1999.

³ Consensus Document on Compositional Considerations for New Varieties of Maize (*Zea mays*): Key Food and Feed Nutrients, Anti-nutrients and Secondary Plant Metabolites. Series on the Safety of Novel Foods and Feeds No. 6 (2002). Environment Directorate: Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology.

6. Regulatory Options

6.1 Option 1 – prohibit food from insect-protected, glyphosate-tolerant corn line 88017

Maintain the *status quo* by not amending the Code to approve the sale and use of food derived from insect-protected, glyphosate-tolerant corn line 88017.

6.2 Option 2 – approve food from insect-protected, glyphosate-tolerant corn line 88017

Amend the Code to permit the sale and use of food derived from insect-protected, glyphosate-tolerant corn line 88017, with or without listing special conditions of use in the Table to clause 2 of Standard 1.5.2.

7. Impact Analysis

7.1 Affected parties

- 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.

In the event that the Applicant proceeds to commercialise MON 88017 corn in the United States agricultural markets in the future, the approval of this application would ensure corn or food imports from the US to Australia and New Zealand would comply with the Code.

There is no current intention to grow MON 88017 corn in Australia or New Zealand. Should this be decided in the future, any environmental impact would require assessment by the Office of the Gene Technology Regulator (OGTR) in Australia, and by various New Zealand government agencies including the Environmental Risk Management Authority (ERMA) and the Ministry of Agriculture and Fisheries (MAF) before cultivation in these countries could be permitted.

7.2 Impact 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- prohibit food derived from corn line 88017

Consumers: Cost in terms of a possible reduction in the availability of certain food products. Benefit to consumers if there are potential public health and safety issues.

Cost associated with higher retail prices for segregated foods.

No impact on consumers wishing to avoid GM foods, as food from corn line 88017 is not currently permitted in the food supply.

Government: No immediate impact.

In certain circumstances, there could be resource costs associated with monitoring for non-approved GM commodities.

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

Industry: 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.

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 corn line 88017

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

Benefit of access to the full range of imported corn products that potentially could contain ingredients derived from corn line 88017 (after commercialisation of the line).

Cost to consumers wishing to avoid GM food by a potential restriction of choice of products, or increased prices for non-GM food.

Government: No direct impact.

This decision could impact on monitoring resources, as certain foods derived from corn line 88017 will be required to be labelled as genetically modified.

Industry: Potential benefit to importers and wholesalers of overseas food products due to a broad access to products derived from non-segregated corn grain.

Potential benefit to the food industry if reduced use and exposure to agricultural chemicals results in lower production costs that are passed on to wholesalers and other sectors downstream.

Benefit for food manufacturers in that the choice of raw ingredients is not confined to a limited range of GM and non-GM varieties of corn.

Benefit to food retailers in the availability of a broad product range.

Possible cost to food industry as some food ingredients derived from corn line 88017 will be required to be labelled as genetically modified.

8. Consultation

8.1 Public Consultation

The Initial Assessment was advertised for public comment between 15 December 2004 and 5 February 2005. A total of 139 submissions were received during this period and a summary of these is included in **Attachment 3** to this Report. All but five of the submissions were from New Zealand. A significant proportion of these were brief, campaign letters expressing opposition to all GM foods.

FSANZ has taken the submitters' comments into account in preparing the draft assessment of this application. Specific issues relating to corn line 88017 have been addressed in the report.

8.2 World Trade Organization (WTO)

As members of the World Trade Organization (WTO), Australia and New Zealand are obliged 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.

There are no relevant international standards for GM foods, however the proposed amendment to the Code to allow food derived from corn line 88017 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, FSANZ will be recommending to the agencies responsible that the WTO be notified under the Sanitary and Phytosanitary Measure (SPS) Agreement, in order to enable other member nations to comment on the proposed changes to standards that may have a significant impact on them.

9. FSANZ decision

Food derived from insect-protected and glyphosate-tolerant corn line 88017 is as safe for human consumption as food from other commercial corn varieties.

FSANZ recommends that food derived from corn line 88017 be approved.

9.1 Statement of Reasons

An amendment to the Code to give approval to the sale and use of food derived from corn line 88017 (MON 88017) in Australia and New Zealand is proposed 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 insect-resistant and glyphosate-tolerant corn line 88017;
- food derived from corn line 88017 is equivalent to food from other commercially available corn varieties in terms of its safety for human consumption and nutritional adequacy;
- labelling of certain food fractions derived from corn line 88017 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 amendment to the Code is of net benefit to both food producers and consumers.

ATTACHMENTS

1. Draft variation to the *Australia New Zealand Food Standards Code*
2. Draft Safety Assessment Report for corn line 88017
3. Summary of first round public submissions

ATTACHMENT 1

DRAFT VARIATION TO *THE AUSTRALIA NEW ZEALAND FOOD STANDARDS CODE*

To commence: on gazettal

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

Food derived from corn rootworm-protected and glyphosate-tolerant corn line 88017

DRAFT SAFETY ASSESSMENT

APPLICATION A548: FOOD DERIVED FROM CORN ROOTWORM-PROTECTED
AND GLYPHOSATE-TOLERANT CORN LINE 88017

SUMMARY AND CONCLUSIONS

Background

Corn line 88017 (MON 88017) has been genetically modified for protection against the insect pest, corn rootworm, and tolerance to the broad-spectrum herbicide, glyphosate. MON 88017 corn was developed primarily for cultivation in the United States and Canada and is not intended for cultivation in Australia or New Zealand. If approved, food derived from MON 88017 corn would enter the Australian and New Zealand food supply through imported, largely processed, food products only.

In conducting a safety assessment of food derived from MON 88017 corn, a number of criteria have been addressed including: a characterisation of the transferred genes, their origin, function and stability; 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.

History of Use

Corn (*Zea mays L*), otherwise known as maize, is the world's third leading cereal crop, behind wheat and rice, and is grown in over 25 countries worldwide. Corn-derived products are routinely used in a large number and diverse range of foods and have a long history of safe use. Products derived from MON88017 corn may include flour, breakfast cereals, oil, high fructose corn syrup, and other starch products.

Description of the Genetic Modification

MON88017 corn was generated through the transfer of the *cp4 epsps* and *cry3Bb1* genes to an inbred hybrid corn line, A x Hi-II. 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) that continues to function in the biochemical pathway producing aromatic amino acids in a plant that has been sprayed with glyphosate, when the plant's own EPSPS enzyme has been inactivated by the herbicide. The *cry3Bb1* gene is derived from the soil bacterium *Bacillus thuringiensis* subspecies *kumamotoensis* and encodes the Cry3Bb1 protein which is selectively toxic to certain Coleopteran insects in the larval stage. The DNA sequence of the *cry3Bb1* gene transferred to MON 88017 is a close variant of the native gene sequence and has been designed to encode the Cry3Bb1 variant protein with enhanced insecticidal activity against corn rootworm. There was no transfer of bacterial antibiotic resistance marker genes in this modification.

Detailed molecular and genetic analyses of MON 88017 corn indicate that the transferred genes are stably integrated into the plant genome as single linked copies at the same insertion site, and are inherited in subsequent generations according to predicted patterns of inheritance.

Characterisation of Novel Protein

Two novel proteins are expressed in MON88017 corn – CP4 EPSPS and the Cry3Bb1 variant protein. The mature CP4 EPSPS in MON88017 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 Cry3Bb1 in MON88017 differs from the native Cry3Bb1 by six amino acid changes which were deliberately introduced. The Cry3Bb1 protein is also present in an approved line of corn known as MON863. The variant in MON88017 differs from that in MON863 corn, approved in 2003, by only one amino acid.

Both novel proteins are expressed at relatively low levels in MON88017 plants. The mean level of CP4 EPSPS in grain was 5.8 µg/g dry weight, with concentrations in other plant tissues ranging from 390-57 µg/g dry weight. In general, the level of CP4 EPSPS protein declined during the growing season. The mean Cry3Bb1 protein level in the grain was 15 µg/g dry weight, with concentrations in other tissues ranging from 570-25 µg/g dry weight.

Potential toxicity and allergenicity

The two novel proteins present in MON88017 corn have been assessed previously for safety; the CP4 EPSPS protein is present in approved lines of canola, cotton, soybean, potato and other corns. Previous assessments have shown that CP4 EPSPS administered directly to animals at a high dose is not toxic, and the evidence indicates no potential for this protein to be allergenic in humans. Given its widespread use in approved glyphosate-tolerant crops, it now has a history of safe use in food over 10 years.

In considering the potential toxicity and allergenicity of the Cry3Bb1 variant protein, it is worth noting that *Bt* formulations containing Cry3Bb1 have been used safely in agriculture since 1995. Two separate acute toxicity studies in mice using the individual Cry3Bb1 variant proteins present in MON 88017 and MON 863 respectively confirmed the absence of mammalian toxicity in each case. Bioinformatic studies confirmed the absence of any significant amino acid similarity with known protein toxins and allergens, and *in vitro* digestibility studies demonstrated that Cry3Bb1 variants are rapidly degraded in the stomach following ingestion. Furthermore, processing involving heat treatment renders the Cry3Bb1 variant protein non-functional (i.e. unable to exert a toxic effect in insects). This weight of evidence indicates that the Cry3Bb1 variant protein is not toxic and is unlikely to pose an allergenic risk to humans.

Comparative Analyses

Compositional studies were conducted to establish the nutritional adequacy of MON88017 corn compared to the non-GM control line and conventionally produced commercial corn varieties. The constituents measured were proximate analysis, fatty acids, amino acids, vitamins, minerals, secondary metabolites, and anti-nutrients such as phytic acid.

In general, no differences of biological significance were observed between MON 88017 corn and its non-GM counterpart. A reduction of approximately 23% in vitamin B₁ levels was observed in MON 88017, however the levels were well within the literature range for this nutrient in conventionally bred corn. As vitamin B₁ levels in corn are generally low, a reduction of this magnitude is not considered to be nutritionally significant, especially as human food. Other minor differences in fatty acid or amino acid constituents were not indicative of an overall pattern of change that could be attributed to the modification. Grain from MON 88017 corn is therefore considered to be compositionally equivalent to grain from other commercial corn varieties.

Nutritional Impact

The detailed compositional studies are considered adequate to establish the nutritional adequacy of food derived from MON 88017 corn. Nevertheless, two animal feeding studies were considered as supplementary information – one in rats and one in catfish. These studies show no differences in the typical growth and health of the animals fed on diets containing MON 88017 corn grain, compared to animals fed on a suitable control diet. The introduction of MON 88017 corn into the food supply is therefore expected to have minimal nutritional impact.

Conclusion

No potential public health and safety concerns have been identified in the comprehensive assessment of MON 88017 corn. On the basis of the data provided in the present application, and other available information, food derived from MON 88017 corn is considered as safe and wholesome as food derived from other corn varieties.

1. INTRODUCTION

Monsanto Australia Ltd is seeking approval in Australia and New Zealand for insect-protected and herbicide-tolerant corn line 88017 (MON 88017) under Standard 1.5.2 – Food Produced Using Gene Technology in the *Food Standards Code*. MON 88017 has been genetically modified (GM) for protection against corn rootworm and for tolerance to the broad-spectrum herbicide glyphosate.

Corn plants are susceptible to damage from the feeding of a range of insect pests including corn rootworm (*Diabrotica* spp.). Corn line 88017 expresses a variant of the Cry3Bb1 protein isolated from the common soil bacterium *Bacillus thuringiensis* (*Bt*) subspecies *kumamotoensis*. This Bt protein is toxic to specific Coleopteran insects, including three significant pests of corn: Western corn rootworm (*Diabrotica vigifera*), Northern corn rootworm (*Diabrotica berberis*) and Mexican corn rootworm (*Diabrotica vigifera zea*).

The applicant previously developed corn line MON 863, which has been genetically modified (GM) for protection against corn rootworm. Traditional breeding techniques were subsequently used to cross this line with another GM inbred line, expressing tolerance to the broad-spectrum herbicide glyphosate. However, using traditional breeding methods to introduce both traits is considered inefficient and time consuming and therefore MON 88017 corn was developed using a two-gene insertion event that simultaneously creates a single variety of corn containing both agronomic traits.

The glyphosate tolerance trait in MON 88017 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 the activity of the enzyme and resulting in a lack of aromatic amino acids in cells, 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.

Corn, together with rice and wheat, is one of the most important cereal crops in the world with total production of 591 million tonnes in 2000 (FAOSTAT Database 2001). The majority of grain and forage derived from maize is used in animal feed. Maize grain is also used for industrial products such as ethyl alcohol and highly refined starch. Domestic production of corn in Australia and New Zealand is supplemented by the import of a small amount of corn-based food ingredients, largely high-fructose corn syrup, which is not currently manufactured in either country. Corn products are processed into breakfast cereals, baking products, extruded confectionery and corn chips. Other food ingredients such as oils and cornstarch are also imported and used by the food industry for the manufacture of dessert mixes and canned foods.

2. HISTORY OF USE

2.1 Donor Organisms

Bacillus thuringiensis

The use of the bacterium *Bacillus thuringiensis* (*Bt*) as a donor organism has previously been evaluated for safety by FSANZ in relation to GM crops such as cotton, corn and potato. Many different subspecies of *Bt* have been isolated from dead or dying insects, mostly from the orders Coleoptera, Diptera and Lepidoptera, but many subspecies have also been found in the soil, aquatic environments and other habitats (WHO, 1999). The source of the *cry3Bb1* gene used in MON 88017 corn is the *Bt* subspecies *kumamotoensis* which is a spore-forming, gram-positive bacterium that is primarily associated with the soil and leaf surfaces.

The characteristic crystal proteins or inclusion bodies produced by different strains of *Bt* are selectively toxic to certain insects. As the discovery of new crystal proteins continues to rise, current nomenclature, based on amino acid identity, allows closely related proteins to be ranked together. In general, the primary rank of the *Bt* proteins denotes the specific insecticidal activity; for example, Cry1, Cry2, Cry3, and Cry4 proteins are toxic to Lepidopteran, Lepidopteran/Dipteran, Coleopteran, and Dipteran insect pests, respectively (Bravo, 1997; Höfte and Whitely, 1989).

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

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

The report by the WHO International Program on Chemical Safety (WHO, 1999) on *Bt* concludes that ‘*Bt* has not been documented to cause any adverse effects on human health when present in drinking water or food’.

Agrobacterium sp. strain CP4

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 (Padgett *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

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

The commercial production of corn has seen many improvements, particularly since the 1920’s when corn varieties were developed by conventional breeding between progeny of two inbred lines to give hybrid varieties that were known to be superior to open-pollinated varieties in terms of their agronomic characteristics. In present agricultural systems, hybrid corn varieties are used in most developed countries for consistency of performance and production. MON 88017 was derived from an inbred corn line (Hi-II).

Corn has a long history of safe use as food for human consumption, however the amount of whole kernel corn consumed is very small compared to consumption of corn-derived food ingredients. Approximately two-thirds of the grain and forage derived from maize produced in the United States is fed to livestock.

Food uses of corn include the manufacture of corn flakes, baking products, extruded confectionary, corn chips and processed fractions such as oils and sugar syrups. Corn is an excellent raw material for the manufacture of starch (Anderson and Watson, 1982). Approximately one-quarter of cornstarch produced is sold as starch products while the remaining three-quarters is converted to a variety of sweetener and industrial fermentation products including high fructose corn syrup and ethyl alcohol (Watson, 1988; NCGA, 2003; Anderson and Watson, 1982; White and Pollack, 1995). Cornstarch is used by the food industry for the manufacture of dessert mixes and canned foods. In addition to milling and fermentation products, the maize germ can be commercially processed to obtain corn oil and a number of other products (White and Pollak 1995).

Each of these processed corn products may be used as a component of many foods including bakery and dairy goods, beverages, confectionery and meat products.

3. DESCRIPTION OF THE GENETIC MODIFICATION

3.1 Method used in the Genetic Modification

MON 88017 was produced by *Agrobacterium*-mediated transformation of immature embryos of A x Hi-II corn tissue using the transformation vector PV-ZMIR39. Hi-II is a derivative of the A188 and B73 inbred lines of corn, which are publicly available inbred lines from the University of Minnesota and Iowa State University respectively. Hi-II consists of approximately equal proportions of the two lines (Armstrong *et al.*, 1991).

Agrobacterium tumefaciens strain ABI 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. PV-ZMIR39 contains both the left and right transfer-DNA (T-DNA) border sequences to facilitate transformation.

Following inoculation with *Agrobacterium* containing the plasmid, the immature embryos were transferred to a co-culture medium for one to three days so that both the corn immature embryos and *Agrobacterium* were able to grow together so the transformation of individual cells could occur. The process involves the attachment of the bacterium to the corn cells, which leads to transfer of the region of DNA between the left and right borders of the binary plasmid (the T-DNA) into the corn genomic DNA.

Following this incubation period, the immature embryos were transferred to selection medium containing carbenicillin to eliminate *Agrobacterium*, and glyphosate to eliminate those plant cells that were not transformed, so that only cells containing the T-DNA survived. The resulting transformed cells were then subcultured several times on a selection medium and regenerated into plants according to the protocol described by Armstrong and Phillips (1988).

Plants were subsequently screened for insect resistance, glyphosate tolerance, and field performance. Of the many transformation events screened, MON 88017 was selected as the leading commercial candidate.

3.2 Genetic elements in vector

Plasmid PV-ZMIR39 is a disarmed, binary *Agrobacterium tumefaciens* transformation vector that contains both the left and right T-DNA border sequences necessary for insertion into the plant genomic DNA. The T-DNA region in PV-ZMIR39 contains the *cry3Bb1* and *cp4-epsps* genes (highlighted) and regulatory elements necessary for expression in plants, as shown in Table 1.

Table 1: Genetic elements in plasmid PV-ZMIR39

Genetic element	Size (bp)	Function
LEFT BORDER	23	Left border sequence essential for T-DNA transfer (Barker <i>et al.</i> , 1983)
P-ract 1	933	Promoter from rice actin gene (McElroy <i>et al.</i> , 1990)
ract 1 intron	460	Intron from rice actin gene (McElroy <i>et al.</i> , 1991)
CTP2	227	Chloroplast transit peptide from Arabidopsis thaliana (Klee <i>et al.</i> , 1987)
cp4 epsps	1367	Coding sequence for the native CP4 EPSPS enzyme from Agrobacterium sp. strain CP4 (Padgett <i>et al.</i> , 1996)
NOS3'	255	3' untranslated region of the nopaline synthase (NOS) coding sequence, which terminates transcription and directs polyadenylation (Bevan <i>et al.</i> , 1983).
Intervening sequence	32	Synthetic polylinker sequence
P-e35S	612	Promoter from the cauliflower mosaic virus (CaMV) with the duplicated enhancer (Odell <i>et al.</i> , 1985; Kay <i>et al.</i> , 1987)
wt CAB leader	70	5' untranslated region of the wheat chlorophyll a/b-binding protein (Lamppa <i>et al.</i> , 1985)
ract 1 intron	460	Intron from rice actin gene (McElroy <i>et al.</i> , 1991)
cry3Bb1	1961	Coding sequence for a synthetic variant of the Cry3Bb1 protein from <i>Bacillus thuringiensis</i> subsp. <i>kumamotoensis</i> (Romano, C.P. 2002)
tahsp17 3'	233	3' untranslated region of the coding sequence for wheat heat shock protein 17.3 (McElwain and Spiker, 1989)s
RIGHT BORDER	24	Right border sequence essential for T-DNA transfer (Depicker <i>et al.</i> , 1982)

3.2 Function and regulation of novel genes

The two novel genes introduced into MON 88017 corn are:

(i) *cry3Bb1*

Expression of the *cry3Bb1* gene in MON 88017 confers resistance to corn rootworm in the plants. The gene is under the control of the enhanced 35S promoter derived from the plant virus CaMV, which gives rise to constitutive gene expression. The DNA sequence of the *cry3Bb1* gene was synthesised in the laboratory so that codon usage would conform more appropriately with optimal levels of protein expression in plant cells.

(ii) *cp4 epsps*

Expression of the *cp4 epsps* gene in the corn 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.

In MON 88017, the use of the actin promoter derived from rice directs a high level of constitutive expression of the *cp4 epsps* gene in corn, conferring tolerance to the herbicide at the whole plant level. The chloroplast transit peptide sequence (CTP2) was necessary to direct the newly translated CP4 EPSPS protein to the plant chloroplasts, where the endogenous plant EPSPS is functionally active. 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 and sugarbeet, as well as a number of other GM corn varieties.

3.2 Characterisation of the Genes in the Plant

Studies submitted:

Beasley, K.A., Anderson, H.M., Wimberley, P.B., Mittanck, D.W. and Lirette, R.P. (2002). Molecular Analysis of YieldGard® Rootworm/Roundup Ready® Corn Event MON 88017. Monsanto Company Study 01-01-50-11, MSL-17609

Analysis of the DNA introduced into MON 88017 corn was undertaken using a range of established molecular techniques. Southern blot analyses were performed on genomic DNA extracted from MON 88017 corn grain and a similar conventional corn as control to assess the following:

- (i) Number of insertions of the integrated expression cassettes;
- (ii) Number of copies of the integrated expression cassettes;
- (iii) Integrity of gene expression cassettes; and
- (iv) Stability of the inserted DNA with conventional breeding over several generations.

The Southern blot hybridisations (based on the method described by Southern, 1975) involved both short (3-5 hours) and long (14-17.5 hours) gel runs in order to improve the resolution of different size molecular fragments.

Individual Southern blots were tested with probes corresponding to the *cp4 epsps* and *cry3Bb1* genes of interest and their respective promoters, introns and polyadenylation sequences, and the transforming plasmid backbone. In all, 12 separate radiolabelled probes corresponding to segments of DNA spanning the entire length of the plasmid pV-ZMIR39 were used in the analyses.

The primary test substance was genomic DNA isolated from the grain of MON 88017 generation [LH198BC0F₁ x LH59] F₂ (see Figure 2). The control substance was a non-transgenic corn hybrid representing the genetic background of MON 88017.

An additional control from the breeding tree of corn event MON88017, the non-transgenic corn line LH198, was used in the insert stability analysis.

The combined results from these multiple Southern blot analyses establish that MON 88017 is characterised by the presence of one copy of the gene cassette (carrying the 2 genes of interest), inserted at a single locus in the corn genome. No unexpected hybridisation bands were detected. These results indicate that corn event MON 88017 does not contain any additional DNA elements other than those expected from the insertion of the two-gene expression cassette. Fragments corresponding to partial genes, regulatory elements or backbone sequences derived from the transforming plasmid were not detected in MON 88017 corn.

PCR analyses

The organisation of the elements within the insert in MON 88017 was confirmed using polymerase chain reaction (PCR) to amplify seven overlapping regions of DNA that span the entire length of the insert and short flanking regions in the corn genome. Amplification of genomic DNA extracted from MON 88017 as well as the plasmid PV-ZMIR39, generated the expected size PCR products. The generation of the predicted size PCR products from MON 88017 correlated with the results obtained in the Southern analyses and confirmed that the arrangement or linkage of genetic elements in the corn plants is the same as those in the transforming plasmid.

DNA sequence analysis

All seven of the PCR products generated from MON 88017 were subjected to DNA sequencing to provide more detailed information on the integrity of the genetic elements inserted into the plant, and to determine whether any changes in the nucleotide sequence had occurred.

The inserted DNA consists of 7126 base pairs (bp), beginning at base 12072 of plasmid PV-ZMIR39, located in the left border sequence, and ending at base 6829 in the polylinker sequence immediately after the tahsp17 3' polyadenylation sequence of the plasmid. In addition, 103 bp of corn genomic DNA flanking the 5' end of the insert and 221 bp of corn genomic DNA flanking the 3' end of the insert were also reported.

A linear map of the inserted DNA in corn event MON 88017 is presented below. According to the Southern hybridisation analyses and the results of DNA sequencing across the entire insert and into plant genomic DNA at both ends of the insert, the arrangement of genetic elements in the plant correlates exactly with those present in the transforming plasmid PV-ZMIR39.

Figure 1: Schematic representation of the gene elements introduced into MON 88017 corn

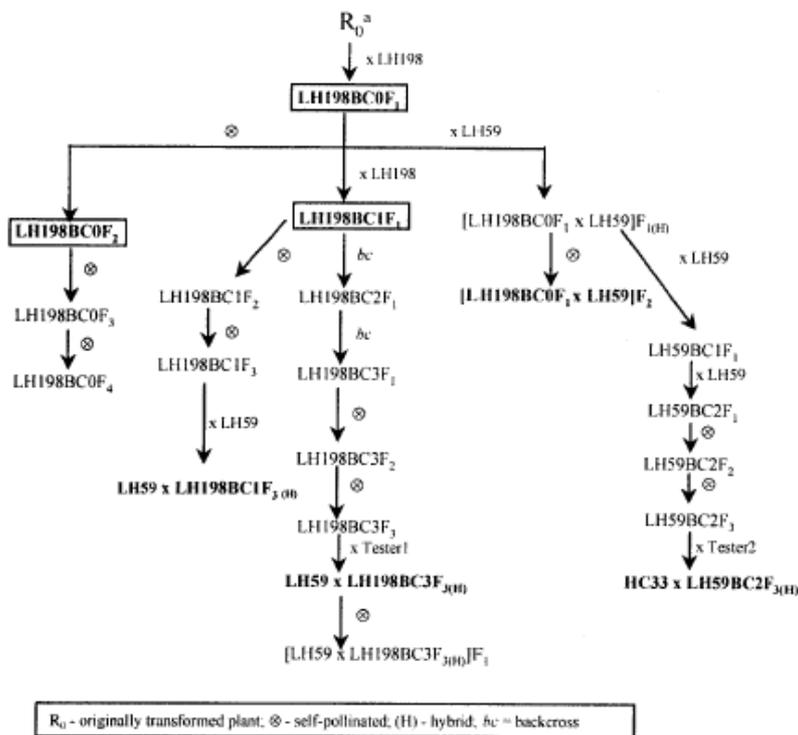


3.3 Stability of the Genetic Changes

In order to demonstrate the stability of the genetic change over multiple generations, additional copy number Southern analyses were performed on six generations from the breeding tree of corn event MON 88017. The specific generations tested are indicated in bold in Figure 2. The [LH198BC0F₁ x LH159]F₂ generation, which was used for the molecular characterisation analyses, was used as reference in this analysis.

Six generations of MON 88017 corn produced the same size molecular bands as in the previously characterised generation. These results, showing that the expected pattern of bands for MON 88017 was maintained across the branches of the breeding tree, demonstrate that the integrated DNA in corn event MON 88017 is stable over multiple generations of conventional breeding.

Figure 2: Breeding tree of corn event MON 88017



Data	MON 88017 Generation
Molecular analysis:	[LH198BC0F ₁ x LH159]F ₂
Insert stability (Southern. blot):	generations bolded
2002 T/C/R trials:	LH59 x LH198BC3F _{3(H)} seed; [LH59 x LH198BC3F _{3(H)}]F ₁ grain
Expression/composition:	LH59 x LH198BC3F _{3(H)} seed; [LH59 x LH198BC3F _{3(H)}]F ₁ grain

Segregation data

Phenotypic data over ten generations was also examined to establish heritability and stability of the genetic change in MON 88017. Expected and observed segregation frequencies of MON 88017 progeny positive for the corn rootworm-protected (*cry 3Bb1*) and glyphosate-tolerant (*cp4 epsps*) phenotypes were recorded and subjected to chi square analysis.

Plants were positively identified as corn rootworm-protected by detection of the Cry3Bb1 protein using ELISA.

The stability of the inserted DNA in MON88017 corn was demonstrated through seven generations of cross-fertilisation and three generations of self-pollination. In general, the results of the statistical analysis are consistent with a single locus of insertion of the *cry 3Bb1* and *cp4 epsps* genes segregating according to predicted Mendelian patterns of inheritance.

3.4 Antibiotic Resistance Genes

The molecular characterisation shows that the region outside of the T-DNA of plasmid PV-ZMIR39 was not integrated into the corn genome during transformation, and therefore genetic elements located on the plasmid backbone were not transferred to the plants. Consequently, the bacterial selectable marker gene, *aad* (which confers resistance to the antibiotics spectinomycin and streptomycin), is not present in corn line MON 88017. The absence of the bacterial marker gene in the plant was confirmed by the Southern hybridisation analysis using a probe specific for detecting the *aad* gene.

4. CHARACTERISATION OF NOVEL PROTEINS

4.1 Function and phenotypic effects

The expression of two novel proteins in MON 88017 confers two new phenotypic traits – protection from corn rootworm damage and tolerance to glyphosate.

Insect protection

The Cry3Bb1 protein is a member of the Cry3Bb class of insecticidal proteins that share >95% amino acid sequence homology. Wild-type Cry 3Bb1 consists of 652 amino acid residues (Donovan *et al.*, 1992).

Corn line MON 88017 expresses a variant of the wild-type Cry3Bb1 protein isolated from *Bacillus thuringiensis* (subsp. *kumamotoensis*) strain EG4691. The variant consists of 653 amino acid residues due to an additional residue (alanine) at position 2 in the protein, which resulted from the need to create a DNA restriction enzyme site for laboratory manipulations. In addition, there are five other specified amino acid changes in the protein expressed in the corn compared to the wild-type Bt protein. The changes amount to 99.1% amino acid identity of the Cry3Bb1 variant with the wild-type Cry 3Bb1 protein.

The amino acid sequence of the Cry3Bb1 variants present in MON 88017 and a previously assessed line, MON 863 (YieldGard® Rootworm corn), share 99.8% identity; they differ by only one of 653 amino acid residues.

Herbicide tolerance

Expression of the CP4 EPSPS protein in MON 88017 corn 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.

The mature 47.6 kDa CP4 EPSPS protein consists of a single polypeptide of 455 amino acids. In MON 88017, the pre-protein consists of 531 amino acids including the CTP2 transit peptide of 76 amino acids, which is cleaved on uptake into the plant chloroplasts.

4.2 Protein Expression Analysis

Studies submitted:

Bhakta, N.S., A.J. Hartmann and J.C. Jennings. Cry3Bb1 and CP4 EPSPS Protein Levels in Corn Tissues Collected from MON 88017 Corn Produced in U.S. Field Trials Conducted in 2002. Monsanto Report MSL Number –18823, completed

The levels of the CP4 EPSPS and Cry3Bb1 proteins in various tissues of MON 88017 plants were measured using an enzyme-linked immunosorbent assay (ELISA) system specifically developed for each protein. For detection of CP4 EPSPS, mouse monoclonal antibodies were raised; quantitation of protein levels was accomplished by interpolation from a CP4 EPSPS protein standard curve that ranged in concentration from 0.456 – 14.6 ng/ml. For detection of Cry3Bb1, goat polyclonal antibodies were raised; quantitation of protein levels was accomplished by interpolation from a Cry3Bb1 protein standard curve that ranged in concentration from 0.35 – 11.2 ng/ml.

To produce the material for analysis, MON 88017 and conventional corn hybrids were planted at three field locations during the growing season in 2002. The sites were located in major corn-growing regions in the US – Iowa, Illinois and Nebraska. Tissues collected at appropriate times during the growing season included pollen, silk, forage root, grain, stover (above-ground material after harvest) and young leaf.

The grain samples had the lowest mean levels of the CP4 EPSPS and Cry 3Bb1 proteins of all plant tissues measured; the levels were 5.8 µg/g dwt (dry weight) and 15 µg/g dwt respectively. Compared to young leaf tissue, which had 220 µg/g dwt of CP4 EPSPS and 570 µg/g dwt of Cry3Bb1, the levels in grain were very low. In addition, levels of the CP4 EPSPS protein declined during the growing season.

4.3 Characterisation of the novel proteins in MON 88017

4.3.1 CP4 EPSPS protein

Studies submitted:

Bonner, H., Ganguly, T., Vaughn, A., Lee, J. and Hileman, R.. Assessment of the Physicochemical and Functional Equivalence of the CP4 EPSPS Protein Produced in Grain of MON 88017 Corn and the *E. coli*-produced CP4 EPSPS Protein. Monsanto Report MSL Number: 18815, completed November 2003.

Quantities of the CP4 EPSPS protein are produced as reference material by expression in *E. coli*. This microbially-produced reference material is used in toxicity and allergenicity studies and to establish that the CP4 EPSPS protein isolated from the grain of MON 88017 corn plants corresponds biochemically to the reference material produced in the laboratory. A panel of analytical tests was used to identify, characterise and compare the plant- and microbially-produced CP4 EPSPS proteins.

The tests included:

- (1) Western blot analysis and densitometry;
- (2) sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and densitometry;
- (3) matrix-assisted laser desorption ionization time of flight (MALDI-TOF) mass spectrometry (MS);
- (4) N-terminal sequence analysis;
- (5) glycosylation analysis; and
- (6) CP4 EPSPS activity assay.

The results are summarised as follows:

- (1) Western blot analysis – a protein band migrating at an approximate molecular weight of 45 kDa on SDS-PAGE gel was identified as the CP4 EPSPS protein using a goat anti-CP4 EPSPS antibody. This serum previously was shown to be specific for the CP4 EPSPS protein. One immunoreactive band migrating at approximately 45 kDa was observed in both the *E. coli*- and plant-produced CP4 EPSPS samples, confirming the identity of the CP4 EPSPS protein produced in MON 88017.
- (2) Molecular weight and purity – The apparent molecular weight of the plant-produced CP4 EPSPS protein, estimated by comparison to molecular weight markers on a stained gel, was 45 kDa (calculated as the average of 3 loadings). The plant- and *E. coli*-produced proteins co-migrated on the gradient gel. The difference in estimated molecular weights between the plant- and *E. coli*-produced CP4 EPSPS proteins was 3%. The purity of the plant-produced CP4 EPSPS protein was calculated at 45.5%.
- (3) MALDI-TOF – The average mass determined from three separate spectral acquisitions was 47447.25 Da. This value compares well with the predicted mass for CP4 EPSPS as 47730 Da, based on the deduced amino acid sequence.
- (4) N-terminal sequence – The observed amino acid sequence for a stained protein band migrating at 45 kDa from the plant-produced CP4 EPSPS sample, matched the expected N-terminal amino acid sequence of the CP4 EPSPS protein over residues 2-18.
- (5) Glycosylation – Using the *E. coli*-produced protein as a negative control and transferrin as a positive control, analysis for covalently bound carbohydrate moieties did not indicate that the plant-produced CP4 EPSPS protein was glycosylated.
- (6) Enzyme activity – The specific activities of the *E. coli*-produced and plant-produced CP4 EPSPS proteins were similar using a phosphate release assay.

Based on the similarity of the results from the plant and microbial preparations, the CP4 EPSPS protein expressed in MON 88017 corn is equivalent to the *E. coli*-produced protein.

4.3.2 *Cry3Bb1* protein

Studies submitted:

Bonner, H.K.S., T. Ganguly, A.P. Vaughn, J.L. Lee and R.E. Hileman. Assessment of the Physicochemical and Functional Equivalence of the Cry3Bb1.pvzmir39 Protein Produced in Grain of MON 88017 Corn and the *E. coli*-Produced Cry3Bb1.pvzmir39 Protein. Monsanto Report MSL Number: 18816, completed 2003.

A panel of analytical tests was used to identify, characterise and compare the Cry3Bb1 protein produced in MON88017 corn plants to that produced from *E. coli* cultures that express the Cry3Bb1 protein and protease-cleaved products of Cry3Bb1.

The Cry3Bb1 protein (predicted molecular weight of 74.4 kDa) is processed in corn to produce peptides with apparent molecular weights of ~66 and ~55 kDa. The ~55 kDa peptide is thought to correspond to the trypsin core of the protein described in the literature (Schnepf *et al.*, 1998).

The analytical tests included:

- (1) Western blot analysis and densitometry;
- (2) SDS-PAGE and densitometry;
- (3) MALDI-TOF mass spectrometry (MS);
- (4) N-terminal sequence analysis;
- (5) glycosylation analysis; and
- (6) a Cry3Bb1 bioactivity assay.

The results are summarised as follows:

- (1) Western blot analysis – Using goat anti-Cry3Bb1 serum, four immunoreactive bands migrating at approximately 77, 66, 55, and 46 kDa were observed in the sample purified from MON 88017 grain. The 46 kDa band is considered to represent a degradation product of Cry3Bb1 protein extracted from corn.
- (2) Molecular weight and purity – The estimated molecular weight from the gradient gel of the full-length Cry3Bb1 protein in the plant-produced sample was 77.2 kDa. The apparent molecular weight of the full-length *E. coli*-produced Cry3Bb1 protein, on the same gel, was estimated to be 77.7 kDa.

The molecular weights of the additional major protein bands, previously identified as Cry3Bb1, in the plant-produced sample were estimated to be 66.2 and 55.4 kDa in this system. The purity of the plant-produced Cry 3Bb1 protein was calculated at 66.1%.

- (3) MALDI-TOF – The average mass determined from three separate spectral acquisitions was 47447.25 Da. This value compares well with the predicted mass for CP4 EPSPS as 47730 Da, based on the deduced amino acid sequence.

- (4) N-terminal sequence – The observed amino acid sequence for a stained protein band migrating at 45 kDa from the plant-produced CP4 EPSPS sample, matched the expected N-terminal amino acid sequence of the CP4 EPSPS protein over residues 2-18.
- (5) Glycosylation – Using the *E. coli*-produced protein as a negative control and transferrin as a positive control, analysis for covalently bound carbohydrate moieties did not indicate that the plant-produced CP4 EPSPS protein was glycosylated.
- (6) Enzyme activity – The specific activities of the *E. coli*-produced and plant-produced CP4 EPSPS proteins were similar using a phosphate release assay.

Based on the similarity of the results from the plant and microbial preparations, the CP4 EPSPS protein expressed in MON 88017 corn is equivalent to the *E. coli*-produced protein.

4.4 Potential toxicity of novel proteins

4.4.1 CP4 EPSPS

The CP4 EPSPS protein has previously undergone assessment by FSANZ when present in other GM (glyphosate tolerant) crop varieties including soybean (RoundUp Ready® soybean), cotton, canola, sugarbeet and other lines of corn. The data submitted for an assessment of potential toxicity have therefore been comprehensively appraised (see Final Assessment Reports for FSANZ Applications A338, A346, A355, A363, A378, A383, A416, A525 and A553).

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.

4.4.2 Cry3Bb1

The safety of the Cry3Bb1 protein has previously been considered by FSANZ as a component of the assessment of corn rootworm-protected MON 863 corn (2003)⁴. Additional toxicity studies have been carried out with the Cry3Bb1 protein present in MON 88017, although previous studies on the variant protein in MON 863 are also relevant to this assessment. The results of the more recent protein toxicity study in mice are consistent with the previous study using the MON 863 variant.

Studies submitted:

McCoy, R.L. and Silanovich, A. (2003). Bioinformatics Analysis of the Cry3Bb1 Protein as Expressed in Corn Event MON 88017 Utilizing the AD4, TOXIN5 and ALLPEPTIDES Databases. Monsanto Company, MSL-18709.

Bonnette, K.L. (2003). An Acute Oral Toxicity Study in Mice with *E. coli*- Produced Cry 3Bb1.pvzmir39 Protein. Monsanto Company, Study No. SB-2003-002.

⁴ Food Derived From Insect-Protected MON863 Corn – A Safety Assessment. Technical Report Series No. 34, FSANZ, 2004. ISBN 0 642 34581 3

Previous studies submitted:

Bonnette, K.L. and Pyla, P.D. (2001). An acute oral toxicity study in mice with *E. coli* produced Cry3Bb1.11098(Q349R) protein. Monsanto Company, MSL-17382.

Hileman, R.E., Rice, E.A., Goodman, R.E., Astwood, J.D. (2001). Bioinformatics evaluation of the Cry3Bb1 protein produced in corn event MON863 utilising allergen, toxin and public domain databases.

History of use

Formulations of *B. thuringiensis*, expressing a number of different Cry proteins, have been used safely and effectively over the last 40 years for the control of a wide variety of insect pests. The Cry3 class of proteins, to which Cry3Bb1 belongs, have been registered for use in the United States and other countries for a number of years and formulations containing Cry3Bb1 as one of the active ingredients have been in commercial use in the United States since 1995 (Baum *et al* 1996). The deduced amino acid sequence of the Cry3Bb1 variant protein expressed in MON863 corn is >98.9% identical to the Cry3Bb1 protein contained in the commercialised biopesticide product.

In addition, the Cry3Bb1 protein also shares approximately 67% amino acid identity to Cry3Aa4, which provides control of the Colorado potato beetle and has been used commercially in various insecticidal sprays since 1989.

Specificity

The Cry proteins are a highly specific group of insect toxins. Their toxicity towards Lepidopteran, Dipteran and Coleopteran insect larvae is well documented. A critical step in the mechanism of action of the Cry proteins is their binding to specific receptors in the intestine of the target organism (Wolfersberger 1990, Ferré *et al* 1991). Without such receptor binding, no toxic effect can be exerted. No receptors for the Cry proteins have been identified on the intestinal cells of mammals (Noteborn *et al* 1993), which explains the absence of toxic effects in species other than insects.

Acute oral toxicity study

An acute oral toxicity study using CD-1 mice was conducted to examine the potential toxicity of the Cry3Bb1 protein present in MON 88017 corn. The scientific basis for this approach is that, if toxic, proteins are known to act via acute mechanisms and laboratory animals have been shown to exhibit acute toxic effects from exposure to proteins known to be toxic to humans (Sjogblad *et al* 1992).

It was not possible to isolate sufficient quantities of the Cry3Bb1 variant protein from MON88017 corn for use as the test material in the toxicity study, therefore the test material was produced in the laboratory using an *E. coli* large-scale fermentation system. The equivalence of the *E. coli*- and MON88017 corn-produced proteins was established using a range of methods, including MALDI-TOF mass spectrometry, N-terminal sequencing, immunoblotting, glycosylation analysis and insect bioassay (see Section 4.3.2).

E. coli-produced Cry3Bb1 variant protein was administered by gavage to 10 male and 10 female CD-1 mice as two separate oral doses administered approximately 4 hours apart.

The large target dose of 2442 mg/kg was based on the maximum attainable Cry3Bb1 concentration of the dosing solution (estimated at 37 mg/ml) and a total dose volume of 66.6 ml/kg body weight. The limited solubility of the Cry3Bb1 protein precluded its administration as a single dose. Therefore, dosing was divided into two doses of 33.3 ml/kg body weight to achieve the target dose. On the day of dosing (Day 0), administration of the two individual doses was separated by approximately four hours. A separate control group of ten male and ten female animals received bovine serum albumin (BSA) at a dose of 1900 mg/kg. Analysis of the dosing solutions revealed that the concentration of Cry3Bb1 protein was 79% of target and, therefore, the achieved dose was actually 1930 mg/kg.

Study animals were observed for clinical signs twice on day 0, following each dose of test substance, then daily for 14 days. Clinical observations included, but were not limited to, changes in the skin and fur, eyes and mucous membranes, respiratory system, circulatory system, autonomic and central nervous system, including tremors and convulsions, changes in level of activity, gait and posture, reactivity to handling or sensory stimuli, altered strength and unusual behaviours. A general health/mortality check was performed twice daily. Animals were weighed at the beginning of the study and then weekly thereafter. Food consumption was measured weekly. On day 14 of the study, the animals were killed and examined for gross necropsy and any abnormalities recorded.

No significant differences in food consumption were observed during the study. No animal deaths occurred during the course of the study and no significant clinical abnormalities were noted.

The only notable clinical findings were very minor/transient incidences of rough coat (1/10 test substance males on day 11 only), swelling of the urogenital region (1/10 control females on days 6-14), and urine staining (1/10 test substance females on day 11).

At necropsy, no gross pathological findings related to consumption of Cry3Bb1 protein were observed. For individual animals, the following was recorded:

Test animals: One female was noted as having an enlarged mandibular lymph node. Since this was a single occurrence, it was not considered to be treatment related.

Control animals: Two females exhibited periovarian cysts at the conclusion of the study (day 14), a finding that is commonly observed in this strain of mouse. One female exhibited a subcutaneous abscess that was consistent with the urogenital swelling observed during the in-life phase.

Under the conditions of this study, no evidence of toxicity was observed in any of the animals. The acute oral LD50 of *E. coli*-produced Cry3Bb1 protein is greater than 1930 mg/kg body weight in the mouse.

Summary of previous oral toxicity study

An acute oral toxicity study using CD-1 mice was conducted to examine the potential toxicity of the Cry3Bb1 variant protein as present in MON 863 corn. The amino acid sequences of the Cry3Bb1 variants present in MON 88017 and MON 863 differ by only one of 653 amino acid residues, that is, the two proteins share 99.8% identity. Although conducted separately, the methodology was the same for both studies.

E. coli-produced Cry3Bb1 variant protein was administered by gavage to CD-1 mice (10/sex/group) as two separate oral doses administered approximately 4 hours apart. The dose levels used were 300, 900 and 2700 mg/kg body weight (bw). Bovine serum albumin was again used as a control substance and administered at the dose of 2700 mg/kg bw. Following dosing, the animals were observed daily for 14 days for any clinical signs or mortality. Animals were weighed at the beginning of the study and then weekly thereafter. On day 14 of the study, the animals were killed and examined for gross necropsy and any abnormalities recorded.

No animal deaths occurred during the course of the study and no significant clinical observations were noted. No statistical differences were observed in the body weight or body weight gain data. Slight body weight loss was noted for a few animals, as follows:

Test animals: one male during day 7 to 14 given 2700 mg/kg bw Cry3Bb1 variant protein; one female given 300 mg/kg bw Cry3Bb1 variant protein; one female given 900 mg/kg bw Cry3Bb1 variant protein.

Control animals: one female during day 0 to 7 given the protein control.

These animals however all exceeded their initial body weight by study termination (day 14) and body weight gain was noted for all other animals during the test period. The 300 mg/kg bw dosed males and the 900 mg/kg bw dosed males had a significant increase in food consumption compared to the control group during the 0 to 7 day food consumption interval. No significant gross internal findings were observed at necropsy on day 14. In conclusion, no treatment related adverse effects were observed in the mice when tested with the Cry3Bb1 variant protein at doses up to 2700 mg/kg bw.

Previous assessment of similarities with known protein toxins

Bioinformatic analyses were used to assess whether the Cry3Bb1 variant protein (MON 863) shares sequence similarity with known protein toxins. Protein sequence databases were assembled for this purpose and the FASTA⁵ sequence alignment tool was used to assess structural similarity. Although the FASTA program directly compares amino acid sequences and thus is mainly used to assess primary protein structure, the alignment data may also be used to infer secondary and tertiary structure of proteins.

The extent of similarity was evaluated by visual inspection of the alignment, the calculated percent identity and *E* score value. The *E* score (expectation score) reflects the degree of similarity and the value depends on the overall length of the sequence alignment, the quality (percent identity, similarity) of the overlap and the size of the database. A larger *E* score value indicates a lower degree of similarity between the query sequence (the Cry3Bb1 variant protein amino acid sequence) and the sequence from the database.

As expected, the best similarity observed was to the Cry3Bb1 protein in *B. thuringiensis*, showing 99.1% identity. Inspection of the other sequence alignments between the (TOXIN4) database and the Cry3Bb1 variant protein revealed that almost all of the 169 entries were structurally related sequences from the Cry family of proteins. No other significant sequence similarities with known toxins were detected.

⁵ Algorithm used to find local high scoring alignments between a pair of protein or nucleotide sequences.

Structural similarities between the Cry3Bb1 variant protein and all publicly available proteins were evaluated using the FASTA sequence alignment tool. The best similarity observed was again to the *B. thuringiensis* Cry3Bb1 protein. A further 267 sequence alignments were identified, with 266 of these being structurally related sequences from the Cry family of proteins. The poorest scoring entry corresponded to an uncharacterised protein from *Drosophila melanogaster* (fruitfly).

Previous assessment of heat stability

A study was done to assess the immuno-detectability and bioactivity of the Cry3Bb1 variant protein in MON863 corn following heat treatment of grain similar to that used in the manufacture of corn flakes.

Grain from MON863 corn plants grown in the field and corresponding control lines were ground to a fine powder and baked at 204°C for 30 minutes to simulate the heat step used in food processing.

Samples were then analysed by immunoblotting and ELISA to determine the immuno-detectability of the Cry3Bb1 variant protein. An insect bioassay, using Colorado potato beetle, was also done with the baked and unbaked samples.

Cry3Bb1 variant protein was readily detected in unbaked grain samples using either immunoblot analysis or ELISA. In contrast, Cry3Bb1 could not be detected following heat treatment using either method. These results were also reflected in the insect bioassay, where baked samples exhibited a significant reduction in their insecticidal activity, from 93.75-100% mortality with unbaked sample, reduced to 0-6.25% mortality for baked sample.

These results indicate that processing of corn grain, involving heat treatment, renders the Cry3Bb1 variant protein non-functional.

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.

Using a decision tree approach, when indicated, additional *in vitro* and *in vivo* immunological testing can be conducted. 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 MON 88017 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.

The Cry 3Bb1 protein in MON 88017 is a variant of the wildtype protein isolated from *Bacillus thuringiensis* (subsp. *kumamotoensis*) strain EG4691. *B. thuringiensis* is a spore-forming, gram-positive bacterium that is found naturally in the soil. *B.t.* strains have been used in insecticidal sprays for the past 40 years and, during that period, have not been associated with any reported allergic reactions associated with their use. Humans using the insecticidal sprays have been shown to develop antibodies to the Cry proteins but in no case has the presence of these antibodies been linked with any acute or chronic disease (Nester *et al* 2002).

Similarity to known allergens

Potential structural similarities between the CP4 EPSPS enzyme and proteins in the allergen database were evaluated using the FASTA sequence alignment tool. Inspection of the results showed no significant similarities between the CP4 EPSPS protein and known allergens. No immunologically relevant sequences (identity across eight contiguous amino acids) were detected when the CP4 EPSPS sequence was compared to the AD4 sequence database. Previous bioinformatic analyses of the CP4 EPSPS protein have yielded the same negative results.

Similar analyses of the Cry3Bb1 amino acid sequence identified six proteins with varying but low degrees of similarity to the Cry protein. The best similarity observed was to a protein with only 27.5% identity over a short length of 120 amino acid residues (out of 653 aa). In this listing, the longest stretch of contiguous amino acid identities consisted of only three amino acids. Consequently, no structural and/or functional homology between the Cry3Bb1 protein and this protein can be inferred. Inspection of the remaining five alignments did not show any significant similarities of the Cry3Bb1 protein with known allergens. In addition, no immunologically relevant sequences were detected when the Cry3Bb1 sequence was compared to the AD4 sequence database.

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 (Kimber *et al* 1999; Astwood *et al* 1996; Metcalfe *et al* 1996). 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 analysis⁶.

⁶ Harrison, L.A., Bailey, M.R., Naylor, M.W., Ream, J.E., Hammond, B.G., Nida, D.L., Burnette, B.L., Nickson, T.E., Mitsky, T.A., Taylor, M.L., Fuchs, R.L. and Padgett, S.R. (1996). The expressed protein in glyphosate-tolerant soybean, 5-enolpyruvylshikimate-3-phosphate synthase from *Agrobacterium* sp. strain CP4 is rapidly digested *in vitro* and is not toxic to acutely gavaged mice. *J. Nutr.* **126**, 728-740.

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.

Using the ILSI pepsin digestion protocol, *in vitro* digestibility of Cry3Bb1 in simulated gastric (SGF) and simulated intestinal (SIF) fluids was measured. SGF contains pepsin and SIF contains pancreatin, a mixture of enzymes including amylase, trypsin, lipase, ribonuclease and protease. The rate of digestibility was assessed using Colloidal Brilliant Blue G staining of SDS polyacrylamide gels and Western blots. Virtually all (at least 98-99.8%, depending on the method of detection) of the full-length Cry3Bb1 protein was digested within 15 seconds in SGF.

At least 99.5% of the full-length Cry3Bb1 protein was digested within 1 minute in SIF when evaluated by Western blot analysis. As expected of virtually all Cry proteins, protease-resistant fragments corresponding to the active insect core toxin were observed throughout the 24-hour time course.

The results of the *in vitro* experiments indicate that Cry3Bb1 protein in MON88017 corn is readily digestible in the mammalian digestive tract, and are consistent with previous experiments on the digestibility of the Cry3Bb1 variant protein present in MON863 corn.

4.6 Conclusion

Two novel proteins, CP4 EPSPS and a variant Cry3Bb1, are expressed in MON88017 corn at relatively low levels in most tissues of the plant. When grown under normal field conditions, mean concentrations of the CP4 EPSPS protein range from a high of 220 µg/g fresh weight (fw) in pollen, to a low of 5.1 µg/g fw in the grain. Mean concentrations of the Cry3Bb1 protein range from a high of 76 µg/g fw in young leaves, to a low of 13 µg/g fw in the grain. The mean percentage of total protein in MON88017 grain is approximately 12.5%, and therefore the amounts of CP4 EPSPS and Cry3Bb1 protein in the grain relative to total protein (on a dry weight basis) is calculated at 0.0046% and 0.012% respectively.

The CP4 EPSPS protein is structurally and biochemically similar to other EPSPS enzymes from various plant and microbial food sources (e.g. Baker's yeast) 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 specific lines of soybean, corn, cotton and canola.

A large number of experiments have been done on the Cry3Bb1 protein to study its physicochemical and functional properties, confirm its identity in MON88017 corn, and to determine its potential toxicity and allergenicity. These studies demonstrate that the protein expressed in MON88017 corn conforms in size, amino acid sequence, and immunoreactivity to that expected from the inserted gene sequence, and also exhibits the expected insecticidal activity.

Regarding the potential toxicity and allergenicity of the Cry3Bb1 variant protein, it is worth noting that *Bt* proteins are inherently non-toxic to mammals and have exhibited little tendency to be allergenic to humans over their long history of use. In addition, *Bt* formulations containing the Cry3Bb1 protein have been used safely in agriculture since 1996 in the United States.

A previously assessed acute toxicity study of the Cry3Bb1 variant protein in MON863 corn⁷ found no adverse effects in mice at doses up to 2700 mg/kg bw. An additional toxicity study using the Cry3Bb1 protein in MON88017 similarly found no treatment related toxicity in mice with a single high target dose of 2442 mg/kg bw. It has also been shown that processing, involving heat treatment, renders the Cry3Bb1 protein non-functional (i.e. unable to exert a toxic effect in insects).

Bioinformatic studies confirmed the absence of any significant amino acid or structural similarity with known protein toxins and allergens and digestibility studies demonstrated that the Cry3Bb1 variant protein would be rapidly degraded under gastric conditions following ingestion. The evidence indicates therefore that the Cry3Bb1 protein expressed in MON88017 corn is not a toxic substance and does not pose a likely allergenic risk in humans.

5. COMPARATIVE 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). 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 toxicants/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 components of corn appropriate for this comparative study include protein, fat, carbohydrates, amino acids, fatty acids, vitamins, minerals and phytic acid (OECD 2002).

Field conditions

The composition of forage and grain from MON88017 corn was evaluated and compared to a conventional variety of corn with a similar genetic background (control), as well as to other commercially available corn hybrids. To obtain the relevant plant samples, MON88017 and control corn were grown at three replicated field sites in major corn-growing regions of the US (Iowa, Illinois and Nebraska) during the 2002 field season. To provide additional reference material representative of the agricultural conditions, four commercially available corn hybrids were also grown at each of the test field sites, providing a total of 12 reference comparators.

At each field site, the test, control and reference seeds were planted in a randomised complete block design with three replicates per block. All plants were grown under normal agronomic field conditions for the respective geographical regions and all test plots received an application of glyphosate herbicide according to label directions. Bulk forage samples were harvested at the late dough/early dent stage and bulk grain samples were harvested at maturity.

⁷ Cry3Bb1 expressed in MON863 and MON88017 corn lines differ by one of 653 amino acid residues.

Compositional analyses of the forage samples (above ground parts) included proximates (protein, fat, ash and moisture), acid detergent fibre (ADF), neutral detergent fibre (NDF), minerals (calcium, phosphorous) and carbohydrates by calculation.

Compositional analyses of the grain samples included proximates, ADF, NDF, total dietary fibre (TDF), amino acids, fatty acids (C8-C22), minerals (calcium, copper, iron, magnesium, manganese, phosphorous, potassium, sodium and zinc), vitamins (B₁, B₂, B₆, E, niacin and folic acid), antinutrients (phytic acid and raffinose), secondary metabolites (furfural, ferulic acid, and p-coumaric acid) and carbohydrates by calculation. In all, 68 analytical components of grain were measured however 15 of these were below the limit of quantitation of the assay systems and were therefore excluded from statistical analysis.

The composition of the grain is of particular interest for food safety assessment. Statistical analyses of the compositional data were conducted using a mixed model analysis of variance method. Four sets of comparison were made based on data from each of the three field sites plus data from a combination of all three sites. Statistically significant differences were determined at the 5% level of significance ($p < 0.05$).

Results

The results of the combined site comparisons are presented in Tables 2-7. A summary of the statistically significant differences between MON88017 and the control are presented in Table 8. The results from individual trial sites were also evaluated but are not presented in this report.

The results of the forage and grain analyses showed no statistically significant differences between MON88017 and the non-GM control for 232 of the 248 comparisons conducted. There were no statistically significant differences found in forage, however differences were observed in the following parameters in grain: palmitic acid (16:0), oleic acid (18:1), linolenic acid (18:3), arachidic acid (20:0), copper, methionine, moisture, niacin and serine (one statistical group); linoleic acid (18:2) (three statistical groups); and vitamin B₁ (four statistical groups). Except for vitamin B₁, none of the statistically significant differences were in all four statistical groups (each individual site and the combination of all sites).

Table 2: Amino acid (AA) content in grain from MON 88017 and conventional corn (combined field trials)

Component (% total AA) ^a	MON 88017 Mean ± S.E. (Range)	Control Mean ± S.E. (Range)	Difference (MON 88017 Minus Control)			Commercial (Range) [99% T.I.] ^b
			Mean ± S.E. (Range)	95% C.I. (Lower, upper)	p-Value	
Alanine	7.55 ± 0.084 (7.29 - 7.70)	7.55 ± 0.084 (7.34 - 7.79)	-0.0026 ± 0.039 (-0.19 - 0.18)	-0.097,0.092	0.949	(7.24 - 8.16) [6.66,8.49]
Arginine	4.42 ± 0.11 (4.10 - 4.74)	4.29 ± 0.11 (4.01 - 4.63)	0.13 ± 0.060 (-0.12 - 0.36)	-0.013,0.28	0.066	(3.72 - 5.08) [3.34,5.67]
Aspartic acid	6.22 ± 0.050 (6.09 - 6.34)	6.25 ± 0.050 (6.04 - 6.45)	-0.032 ± 0.067 (-0.34 - 0.18)	-0.20,0.13	0.648	(6.18 - 6.81) [5.77,7.16]
Cystine	2.14 ± 0.054 (1.93 - 2.26)	2.15 ± 0.054 (1.93 - 2.30)	-0.013 ± 0.042 (-0.20 - 0.17)	-0.098,0.073	0.766	(1.82 - 2.58) [1.46,2.89]
Glutamic acid	20.40 ± 0.18 (19.80 - 20.87)	20.44 ± 0.18 (19.91 - 20.84)	-0.036 ± 0.086 (-0.52 - 0.48)	-0.25,0.17	0.686	(19.46 - 21.57) [18.01,22.15]
Glycine	3.45 ± 0.063 (3.32 - 3.62)	3.45 ± 0.063 (3.18 - 3.61)	0.0061 ± 0.031 (-0.081 - 0.19)	-0.058,0.070	0.844	(3.29 - 4.03) [2.81,4.54]
Histidine	2.99 ± 0.049 (2.90 - 3.10)	2.95 ± 0.049 (2.83 - 3.14)	0.032 ± 0.022 (-0.056 - 0.10)	-0.023,0.087	0.200	(2.50 - 3.12) [2.16,3.60]
Isoleucine	3.59 ± 0.037 (3.43 - 3.71)	3.57 ± 0.037 (3.45 - 3.76)	0.025 ± 0.044 (-0.15 - 0.25)	-0.065,0.11	0.577	(3.39 - 3.79) [3.30,3.84]
Leucine	13.28 ± 0.20 (12.69 - 13.62)	13.31 ± 0.20 (12.76 - 14.11)	-0.037 ± 0.098 (-0.69 - 0.56)	-0.28,0.20	0.717	(12.11 - 14.35) [10.72,15.18]
Lysine	2.69 ± 0.058 (2.42 - 2.87)	2.66 ± 0.058 (2.49 - 2.82)	0.024 ± 0.047 (-0.072 - 0.11)	-0.074,0.12	0.614	(2.44 - 3.27) [2.06,3.73]
Methionine	1.98 ± 0.059 (1.85 - 2.05)	2.01 ± 0.059 (1.83 - 2.20)	-0.030 ± 0.043 (-0.15 - 0.12)	-0.14,0.076	0.515	(1.70 - 2.47) [1.37,2.60]
Phenylalanine	5.18 ± 0.059 (4.97 - 5.31)	5.14 ± 0.059 (5.01 - 5.32)	0.035 ± 0.055 (-0.13 - 0.25)	-0.10,0.17	0.545	(4.82 - 5.39) [4.57,5.71]

Component (% total AA) ^a	MON 88017 Mean ± S.E. (Range)	Control Mean ± S.E. (Range)	Difference (MON 88017 Minus Control)			Commercial (Range) [99% T.I.] ^b
			Mean ± S.E. (Range)	95% C.I. (Lower, upper)	p-Value	
Proline	9.39 ± 0.094 (9.02 - 9.69)	9.34 ± 0.094 (8.85 - 9.80)	0.046 ± 0.11 (-0.61 - 0.71)	-0.18,0.27	0.676	(8.35 - 9.72) [7.60,10.37]
Serine	4.83 ± 0.049 (4.65 - 5.04)	4.91 ± 0.049 (4.63 - 5.13)	-0.081 ± 0.068 (-0.47 - 0.42)	-0.22,0.059	0.244	(4.81 - 5.23) [4.60,5.43]
Threonine	3.22 ± 0.040 (3.10 - 3.38)	3.25 ± 0.040 (3.06 - 3.37)	-0.026 ± 0.045 (-0.25 - 0.24)	-0.12,0.067	0.572	(2.96 - 3.55) [2.89,3.84]
Tryptophan	0.54 ± 0.027 (0.48 - 0.60)	0.55 ± 0.027 (0.41 - 0.68)	-0.0090 ± 0.018 (-0.17 - 0.096)	-0.046,0.028	0.627	(0.44 - 0.83) [0.36,0.77]
Tyrosine	3.35 ± 0.16 (2.35 - 3.66)	3.43 ± 0.16 (2.58 - 3.66)	-0.079 ± 0.23 (-1.18 - 0.98)	-0.61,0.46	0.743	(2.26 - 3.80) [2.62,4.26]
Valine	4.79 ± 0.039 (4.60 - 4.92)	4.74 ± 0.039 (4.60 - 4.94)	0.043 ± 0.052 (-0.25 - 0.26)	-0.064,0.15	0.414	(4.44 - 5.04) [4.22,5.27]

S.E.= standard error of the mean; C.I.= confidence interval; T.I.= tolerance interval

^bWith 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

Table 3: Comparison of the fatty acid (FA) content in grain from MON 88017 and conventional corn (combined field sites)

Component (% total FA)	MON 88017 Mean ± S.E. (Range)	Control Mean ± S.E. (Range)	Difference (MON 88017 Minus Control)			Commercial (Range) [99% T.I.] ^b
			Mean ± S.E. (Range)	95% C.I. (Lower, upper)	p-Value	
16:0 palmitic	10.24 ± 0.43 (10.07 - 10.52)	11.27 ± 0.43 (10.14 - 14.57)	-1.03 ± 0.60 (-4.35 - 0.36)	-2.42,0.37	0.128	(9.29 - 17.81) [6.51,16.50]
16:1 pamtioleic	0.18 ± 0.010 (0.16 - 0.21)	0.18 ± 0.010 (0.16 - 0.22)	-0.0030 ± 0.0064 (-0.029 - 0.025)	-0.019,0.013	0.655	(0.054 - 0.21) [0.0017,0.28]
18:0 stearic	2.01 ± 0.073 (1.80 - 2.19)	2.07 ± 0.073 (1.76 - 2.23)	-0.052 ± 0.046 (-0.28 - 0.25)	-0.15,0.042	0.266	(1.68 - 2.30) [1.41,2.53]
18:1 oleic	22.74 ± 0.23 (22.20 - 23.53)	22.87 ± 0.23 (21.43 - 23.51)	-0.13 ± 0.24 (-0.94 - 1.13)	-0.71,0.46	0.613	(19.79 - 34.46) [9.25,44.14]
18:2 linoleic	62.85 ± 0.39 (61.86 - 63.72)	61.52 ± 0.39 (59.10 - 63.18)	1.34 ± 0.53 (-0.64 - 4.19)	0.093,2.58	0.038	(51.64 - 64.12) [41.22,74.09]
18:3 linolenic	1.21 ± 0.062 (1.15 - 1.26)	1.32 ± 0.062 (1.19 - 1.77)	-0.11 ± 0.077 (-0.53 - 0.043)	-0.30,0.079	0.205	(0.84 - 1.91) [0.42,1.95]
20:0 arachidic	0.37 ± 0.010 (0.35 - 0.39)	0.38 ± 0.010 (0.35 - 0.41)	-0.0085 ± 0.0032 (-0.028 - 0.0088)	-0.015,-0.0019	0.012	(0.36 - 0.45) [0.31,0.49]
20:1 eicosenoic	0.24 ± 0.0056 (0.23 - 0.26)	0.25 ± 0.0056 (0.24 - 0.26)	-0.0034 ± 0.0034 (-0.019 - 0.019)	-0.010,0.0036	0.323	(0.24 - 0.36) [0.18,0.40]
22:0 behenic	0.15 ± 0.0027 (0.14 - 0.16)	0.15 ± 0.0027 (0.14 - 0.17)	-0.0062 ± 0.0038 (-0.018 - 0.014)	-0.014,0.0016	0.116	(0.074 - 0.24) [0.071,0.25]

S.E.= standard error of the mean; C.I.= confidence interval; T.I.= tolerance interval ^bWith 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero

Table 4: Comparison of the mineral content in grain from MON 88017 and conventional corn (combined field sites)

Component (Units) ^a	MON 88017 Mean ± S.E. (Range)	Control Mean ± S.E. (Range)	Difference (MON 88017 Minus Control)			Commercial (Range) [99% T.I.] ^b
			Mean ± S.E. (Range)	95% C.I. (Lower, upper)	p- Value	
Calcium (% dwt)	0.0054 ± 0.00035 (0.0047 - 0.0060)	0.0058 ± 0.00035 (0.0049 - 0.0069)	-0.00040 ± 0.00025 (-0.0013 - -0.00006)	-0.0010,0.00021	0.159	(0.0032 - 0.0060) [0.0017,0.0062]
Copper (mg/kg dwt)	1.73 ± 0.086 (1.48 - 2.05)	1.99 ± 0.086 (1.64 - 2.63)	-0.26 ± 0.12 (-0.95 - 0.41)	-0.54,0.016	0.061	(1.01 - 2.34) [0.17,3.00]
Iron (mg/kg dwt)	21.51 ± 0.59 (20.07 - 22.92)	21.84 ± 0.59 (20.31 - 23.93)	-0.33 ± 0.62 (-2.16 - 2.12)	-1.60,0.93	0.595	(16.42 - 26.03) [12.60,31.26]
Magnesium (% dwt)	0.14 ± 0.0034 (0.13 - 0.15)	0.14 ± 0.0034 (0.13 - 0.16)	-0.0022 ± 0.0044 (-0.024 - 0.018)	-0.011,0.0069	0.618	(0.10 - 0.14) [0.088,0.16]
Manganese (mg/kg dwt)	9.72 ± 0.38 (9.01 - 10.76)	9.37 ± 0.38 (7.55 - 10.44)	0.35 ± 0.38 (-0.39 - 1.56)	-0.57,1.27	0.384	(4.96 - 9.81) [2.45,10.60]
Phosphorus (% dwt)	0.39 ± 0.010 (0.37 - 0.41)	0.39 ± 0.010 (0.36 - 0.43)	-0.0042 ± 0.013 (-0.052 - 0.042)	-0.032,0.023	0.754	(0.28 - 0.41) [0.24,0.44]
Potassium (% dwt)	0.41 ± 0.012 (0.39 - 0.44)	0.42 ± 0.012 (0.38 - 0.47)	-0.0063 ± 0.012 (-0.052 - 0.037)	-0.030,0.018	0.592	(0.29 - 0.43) [0.27,0.48]
Zinc (mg/kg dwt)	24.53 ± 0.98 (22.31 - 27.27)	24.92 ± 0.98 (22.02 - 27.18)	-0.39 ± 0.62 (-3.87 - 1.90)	-1.67,0.89	0.534	(17.15 - 26.18) [13.42,31.37]

^adwt=dry weight; S.E.= standard error of the mean; C.I.= confidence interval; T.I.= tolerance interval

^bWith 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

Table 5: Comparison of the proximates and fibre content in grain from MON 88017 and conventional corn (combined field sites)

Component (Units) ^a	MON 88017 Mean ± S.E. (Range)	Control Mean ± S.E. (Range)	Difference (MON 88017 Minus Control)			Commercial (Range) [99% T.I.] ^b
			Mean ± S.E. (Range)	95% C.I. (Lower, upper)	p-Value	
Ash (% dwt)	1.54 ± 0.077 (1.31 - 1.68)	1.59 ± 0.077 (1.23 - 1.97)	-0.049 ± 0.087 (-0.45 - 0.43)	-0.23,0.13	0.573	(1.04 - 1.86) [0.94,1.73]
Carbohydrates (% dwt)	82.32 ± 0.40 (81.61 - 83.39)	82.33 ± 0.40 (80.67 - 83.62)	-0.019 ± 0.25 (-1.39 - 0.94)	-0.62,0.58	0.940	(81.46 - 86.68) [79.39,89.67]
Fat, total (% dwt)	3.64 ± 0.13 (3.44 - 3.96)	3.79 ± 0.13 (3.53 - 4.36)	-0.16 ± 0.080 (-0.63 - 0.15)	-0.35,0.041	0.100	(2.38 - 4.43) [0.74,6.01]
Moisture (% fw)	11.10 ± 0.99 (9.03 - 13.20)	11.60 ± 0.99 (9.73 - 14.20)	-0.49 ± 0.35 (-1.10 - -0.10)	-1.36,0.37	0.212	(9.15 - 14.90) [4.67,17.56]
Protein (% dwt)	12.51 ± 0.35 (11.63 - 13.00)	12.28 ± 0.35 (11.22 - 13.82)	0.23 ± 0.24 (-0.82 - 1.37)	-0.36,0.82	0.379	(9.26 - 13.37) [6.20,15.35]
ADF (% dwt)	3.77 ± 0.16 (3.31 - 4.40)	3.54 ± 0.16 (2.97 - 4.69)	0.23 ± 0.18 (-0.62 - 1.16)	-0.13,0.59	0.203	(2.39 - 4.89) [1.89,5.23]
NDF (% dwt)	12.44 ± 0.62 (10.99 - 13.58)	11.87 ± 0.62 (10.38 - 14.29)	0.57 ± 0.50 (-1.21 - 2.64)	-0.66,1.79	0.299	(8.41 - 16.54) [3.51,21.65]
TDF (% dwt)	16.24 ± 0.71 (13.57 - 18.64)	15.40 ± 0.71 (13.18 - 17.84)	0.84 ± 0.96 (-2.39 - 4.19)	-1.51,3.20	0.414	(11.80 - 23.04) [5.72,27.10]

^aADF=acid detergent fiber; NDF=neutral detergent fiber; TDF=total dietary fiber; S.E.= standard error of the mean; C.I.= confidence interval; T.I.= tolerance interval

^bWith 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

Table 6: Comparison of the vitamin content in grain from MON 88017 and conventional corn for combined field sites

Component (Units) ^a	MON 88017 Mean ± S.E. (Range)	Control Mean ± S.E. (Range)	Difference (MON 88017 Minus Control)			Commercial (Range) [99% T.I.] ^b
			Mean ± S.E. (Range)	95% C.I. (Lower, upper)	p-Value	
Folic acid (mg/kg dwt)	0.48 ± 0.021 (0.38 - 0.60)	0.48 ± 0.021 (0.42 - 0.59)	0.0012 ± 0.030 (-0.074 - 0.11)	-0.072,0.075	0.969	(0.28 - 0.61) [0.12,0.77]
Niacin (mg/kg dwt)	20.94 ± 1.20 (17.04 - 24.14)	21.75 ± 1.20 (19.08 - 23.92)	-0.81 ± 0.42 (-2.04 - 0.23)	-1.67,0.050	0.063	(14.11 - 27.77) [3.19,34.49]
Vitamin B ₁ (mg/kg dwt)	2.47 ± 0.14 (2.30 - 2.69)	3.24 ± 0.14 (2.99 - 3.60)	-0.77 ± 0.12 (-1.02 - -0.35)	-1.06,-0.48	<0.001	(2.69 - 3.73) [1.96,4.38]
Vitamin B ₂ (mg/kg dwt)	1.10 ± 0.041 (0.98 - 1.22)	1.13 ± 0.041 (0.99 - 1.33)	-0.025 ± 0.037 (-0.17 - 0.14)	-0.12,0.066	0.524	(0.88 - 1.32) [0.67,1.51]
Vitamin B ₆ (mg/kg dwt)	7.16 ± 0.22 (6.57 - 8.06)	7.10 ± 0.22 (5.65 - 8.54)	0.063 ± 0.28 (-1.27 - 2.40)	-0.59,0.72	0.828	(4.93 - 7.24) [4.29,7.84]
Vitamin E (mg/kg dwt)	14.15 ± 1.70 (6.08 - 16.93)	14.07 ± 1.70 (1.74 - 17.77)	0.070 ± 1.46 (-11.15 - 14.39)	-2.93,3.07	0.962	(8.09 - 21.97) [0,29.69]

^adwt=dry weight; Vitamin B₁ =Thiamine; Vitamin B₂ =Riboflavin; Vitamin B₆ =Pyridoxine; S.E.= standard error of the mean; C.I.= confidence interval; T.I.= tolerance interval

^bWith 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

Table 7: Content of secondary metabolites and anti-nutrients in grain from MON 88017 and conventional corn for combined field sites

Component (Units) ^a	MON 88017 Mean ± S.E. (Range)	Control Mean ± S.E. (Range)	Difference (MON 88017 Minus Control)			Commercial (Range) [99% T.I.] ^b
			Mean ± S.E. (Range)	95% CI (Lower, upper)	p-Value	
Ferulic acid (µg/g dwt)	2175.34 ± 46.31 (1986.75 - 2275.48)	2121.05 ± 46.31 (1927.55 - 2339.71)	54.29 ± 49.66 (-200.92 - 347.92)	- 47.14,155.72	0.283	(1717.17 - 2687.57) [1415.19,3173.90]
p-Coumaric acid (g/g dwt)	169.26 ± 7.26 (148.45 - 215.25)	154.83 ± 7.26 (141.41 - 173.24)	14.43 ± 9.88 (-14.72 - 72.55)	-9.75,38.61	0.194	(152.30 - 319.15) [43.13,384.34]
Phytic acid (% dwt)	0.95 ± 0.043 (0.83 - 1.05)	0.89 ± 0.043 (0.72 - 1.03)	0.058 ± 0.056 (-0.15 - 0.24)	-0.058,0.17	0.309	(0.45 - 1.00) [0.28,1.12]
Raffinose (% dwt)	0.17 ± 0.013 (0.14 - 0.20)	0.17 ± 0.013 (0.14 - 0.23)	0.00080 ± 0.0081 (-0.035 - 0.036)	-0.019,0.021	0.924	(0.073 - 0.22) [0,0.32]

^adwt=dry weight; S.E.= standard error of the mean; C.I.= confidence interval; T.I.= tolerance interval

^bWith 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

Table 8: Summary of statistically significant differences in composition of MON 88017 and conventional corn

Tissue/Site/ Component (Units) ^a	Mean MON 88017	Mean Control	Mean Diff. (% of Control Value)	Signif. (p-value)	MON 88017 (Range)	99% Tolerance Interval ^b
Grain						
Iowa						
16:0 palmitic (% total FA)	10.16	12.94	-21.50	0.029	(10.11-10.23)	[6.51, 16.50]
18:2 linoleic (% total FA)	63.25	60.41	4.70	0.017	(62.73-63.72)	[41.22, 74.09]
18:3 linolenic (% total FA)	1.25	1.57	-20.26	0.036	(1.24-1.26)	[0.42, 1.95]
Methionine (% total AA)	2.02	2.16	-6.39	<0.001	(1.96-2.05)	[1.37, 2.60]

Tissue/Site/ Component (Units)^a	Mean MON 88017	Mean Control	Mean Diff. (% of Control Value)	Signif. (p-value)	MON 88017 (Range)	99% Tolerance Interval^b
Grain						
Moisture (% fw)	9.38	9.93	-5.54	0.034	(9.03-9.70)	[4.67, 17.56]
Vitamin B ₁ (mg/kg dwt)	2.54	3.07	-17.37	<0.001	(2.42-2.65)	[1.96, 4.38]
Illinois						
18:1 oleic (% total FA)	22.53	23.29	-3.26	<0.001	(22.50-22.56)	[9.25, 44.14]
18:2 linoleic (% total FA)	63.11	62.15	1.55	0.003	(62.84-63.29)	[41.22, 74.09]
Niacin (mg/kg dwt)	21.10	22.52	-6.30	0.014	(20.39-21.52)	[3.19, 34.49]
Vitamin B ₁ (mg/kg dwt)	2.30	3.10	-25.63	<0.001	(2.30-2.30)	[1.96, 4.38]
Nebraska						
Copper (mg/kg dwt)	1.57	2.21	-28.80	0.023	(1.48-1.68)	[0.17, 3.00]
Serine (% total AA)	4.80	4.97	-3.37	0.042	(4.80-4.81)	[4.60, 5.43]
Vitamin B ₁ (mg/kg dwt)	2.58	3.56	-27.53	<0.001	(2.47-2.69)	[1.96, 4.38]
Combination of all sites						
18:2 linoleic (% total FA)	62.85	61.52	2.17	0.038	(61.86-63.72)	[41.22, 74.09]
20:0 arachidic (% total FA)	0.37	0.38	-2.24	0.012	(0.35-0.39)	[0.31, 0.49]
Vitamin B ₁ (mg/kg dwt)	2.47	3.24	-23.72	<0.001	(2.30-2.69)	[1.96, 4.38]

^adwt=dry weight; fw=fresh weight; AA=amino acids; FA=fatty acids;

^bWith 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

Discussion of results

In a study of this magnitude, a small percentage (approximately 5%) of statistically significant differences are 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, minor changes in the levels of some fatty acids (palmitic, linolenic, oleic acids), some amino acids (methionine, serine), and some vitamins and minerals (niacin, copper) are all in this category. Consequently, these differences, although statistically significant for the individual site, are not considered to be biologically meaningful.

A statistically significant increase in the level of linoleic acid was recorded at two of the field sites, which translated to the combined test site data. However, as presented in Table 9, the magnitude of the difference between MON88017 and the control corn grain is small. Furthermore, the values for both MON88017 (approx. 63%) and its non-GM comparator (approx. 62%) are well within the broad range for this fatty acid (48-66% total fatty acid content), and therefore do not represent a nutritional concern.

Table 9: Comparison of levels of 18:2 linoleic acid at trial sites (% total FA)

Field Site	Mean MON88017	Mean Control	Mean Diff. (% of control)	Signif. (p-value)	MON 88017 (Range)	99% Tolerance Interval ^a
Iowa	63.25	60.41	4.70	0.017	(62.73-63.72)	[6.51, 16.50]
Illinois	63.11	62.15	1.55	0.003	(62.84-63.29)	[41.22, 74.09]
Nebraska	62.19	61.99	0.20	0.692	(61.86-62.54)	-
Combined	62.85	61.52	2.17	0.038	(61.86-63.72)	[41.22, 74.09]

^a With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

Of the three B vitamins measured, only vitamin B₁ values in grain from MON88017 corn were significantly lower than the control across all field sites, with a mean decrease of approximately 24% (range 17-28%) for the combined site data. Vitamin B₁ (thiamine) levels in corn grain are generally low and show a broad literature range (3-8.6 mg/kg dwt), as well as a broad historical⁸ range (0.2-0.33 mg/100g dwt). The mean levels of vitamin B₁ in MON 88017 corn are well within the range expected for corn grain derived from conventionally bred plants. All test values found to be statistically different (p<0.05) between MON 88017 corn and the non-GM control were within the 99% tolerance interval.

Conclusion

The comparative analyses do not indicate any compositional differences of biological significance in the grain or forage derived from MON 88017 corn, compared to the non-GM control when grown in different geographical regions.

⁸ Historical range is obtained from control samples analysed in previous studies conducted by Monsanto Company.

Although a decrease of approximately 24% in vitamin B₁ was reported for all test field sites, the absolute levels were well within the range expected for this nutrient for conventionally produced commercial corn hybrids. The levels of vitamin B₁ in corn grain are generally low and show a three-fold variation in the reported literature range. The decrease observed in MON 88017 grain therefore is not considered to raise any nutritional concerns. Overall, grain derived from MON 88017 corn can be considered equivalent in composition to grain from conventionally produced 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 comparative 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 MON88017 corn, the extent of the compositional and other available data is considered sufficient to establish the nutritional adequacy of the food. Nevertheless, two feeding studies conducted on MON88017 corn have been notified, one in rats and the other in catfish. Summaries of these studies are presented below.

Study 1- Rat Feeding Study

A 90-Day Feeding Study in Rats with MON 88017 Corn Grain
Author: J. B. Kirkpatrick
Monsanto Study Number: MSL-19792, completed April 2005.

The objective of this study was to compare the responses of rats fed grain from MON 88017 corn, to the responses of rats fed a conventional control that has background genetics similar to that of the MON 88017 grain.

The study design included three groups of Sprague-Dawley Crl:CD®(SD)IGS BR rats consisting of 20 rats/sex/group. One group was administered a diet containing approximately 11% (w/w) MON 88017, supplemented with 22% (w/w) control grain. A second group was administered a diet containing approximately 33% (w/w) MON 88017. A concurrent control group was administered a diet containing 33% control grain. These diets were administered *ad libitum* for a minimum of 90 days.

All animals were observed twice daily for mortality and moribundity. Clinical examinations were performed daily and detailed physical examinations were performed weekly. Individual body weights and food consumption were recorded weekly. Clinical pathology evaluations (haematology, serum chemistry and urinalysis) were performed on the first 10 animals/sex/group at the scheduled necropsy date (study week 13). Complete necropsies were conducted on all animals and selected organs were weighed at the scheduled necropsy date. Selected tissues were examined microscopically from all animals fed diets containing 33% control and 33% MON 88017 corn grain.

There were no clinical observations related to the test substance during the course of the study and all animals survived to the scheduled necropsy. Body weights, food consumption and clinical pathology parameters were unaffected by the administration of the test substance. There were no test substance-related effects on organ weights. There were no test substance-related findings under macroscopic or microscopic examination.

In conclusion, feeding of MON 88017 corn grain to rats for 90 consecutive days at concentrations up to 33% of the diet had no adverse effects on the growth or health of the animals.

Study 2 - 8 week feeding study in catfish

Evaluation of MON 88017 as a Feed Ingredient for Channel Catfish.

Authors: M.H. Li and E. H. Robinson

Study Number: NW-2003-131, completed August 2004.

Performing Laboratory: Thad Cochran National Warmwater Aquaculture Centre (NWAC), Mississippi State University, Stoneville, MS.

The objective of this study was to evaluate the nutritional quality of MON 88017, which produces the Cry3Bb1 and CP4 EPSPS proteins. This was assessed by comparing the growth and survival of channel catfish fed a diet containing 35% MON 88017 grain, to that of catfish fed a diet containing either 35% grain from a (genetically similar) conventional control corn, or from four commercial reference varieties.

The compositions of the test, control, and reference substance diets were similar to that of typical channel catfish feeds. The diets were formulated to be isonitrogenous and to contain approximately 32% crude protein. The diets were pelleted and fed twice daily to 100 catfish per treatment group (5 replicates with 20 catfish each) for 8 weeks. Fish in each aquarium were counted and weighed at initiation, 4 weeks, and 8 weeks. Mortality and behavior were recorded daily.

At the end of the study (8 weeks), fillets of five fish from each aquarium (25 fish per treatment group) were collected, pooled, and processed to determine the percentages of crude protein, crude fat, moisture, and ash. Channel catfish fed diets containing MON 88017 grain did not differ in survival, weight gain, feed conversion, behavior, or fillet composition from catfish fed the control or commercial reference substances.

Based on these observations, MON 88017 corn is considered to be nutritionally equivalent to conventional corn varieties when fed to catfish.

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SUMMARY OF FIRST ROUND PUBLIC SUBMISSIONS

A total of 139 submissions were received – 134 from New Zealand, 5 from Australia.

Submissions from New Zealand

- 1. New Zealand Food Safety Authority**
 - agree with the issues identified by FSANZ in the Initial Assessment Report.
- 2. Murray Lane**
 - strongly supports the introduction of GE corn and other GE products in NZ
 - ‘green’ groups are scaremongering
 - all of the evidence points to no harm and many potential benefits
 - The Royal Commission report clearly stated ‘proceed with caution’.
- 3. Tom Atkinson**
 - GE food applications involving the use of glyphosate are not a good idea, but GE high lysine corn is acceptable.
- 4. GE Free New Zealand (Susie Lees and Claire Bleakley)**
 - opposed to the use of GE crops for human food.
 - opposed to any approvals for GE foods without proof of absence of risk to public health through independent long-term testing on animals and human volunteers.
 - FSANZ should adopt the precautionary principle and immediately halt all approvals and withdraw approvals of GE foods already in the food supply, until concerns raised by individuals, the NZIGE and PSRG, and scientists worldwide have been addressed.
 - FSANZ safety assessments are subjective and stakeholder consultations are selective and fail to support the public interest.
 - the New Zealand public has lost all faith in the regulatory farce imposed by FSANZ.
 - the labelling provisions for GM foods in the Code are a farce and allow many GE products to remain on the market unlabelled and unseen.
 - approvals for GE foods do not consider particular cultural concerns.
- 5. J. Ruth Lawson**
 - opposed to the Application because considers that (i) the public is opposed to GM foods; (ii) there is a lack of long-term scientific evidence concerning safety and changes in the organism; (iii) the scientific data are inadequate; (iv) safety concerns for MON863 corn have a direct bearing on how data for MON 88017 should be interpreted.
- 6. Physicians and Scientists for Responsible Genetics (PSRG)**
 - opposed to the Application because of concerns with potential allergic reactions
 - express concerns with the long-term safety of glyphosate, Bt toxin and pesticide resistance
 - express marketing concerns for New Zealand; support Identity Preservation and traceability system
 - have concerns with current labelling.

7. **Linda Bench – GE Free Northland in Food and Environment**
 - opposed to the Application because of concerns about risks to public health and safety
 - support the views of the Physicians and Scientists for Responsible Genetics and the New Zealand Institute of Gene Ecology
 - considers that there has been no investigations to find out whether there is a link between the 2-10 fold increase in food-borne illness in the US (1994-1999) and commercial release of transgenic crops and foods. Cancers should be included in such a study.
8. **T. Vallings**
 - opposed to the Application and expresses the same concerns as PSRG.
9. **Anita Smith**
 - opposed to the Application because of the extreme dangers associated with the use of glyphosate
 - there should be full labelling for GE and organic produce
 - healthy food should be subsidised.
10. **Z. Grammer**
 - opposed to the Application and expresses the same concerns as PSRG, and GE Free NZ.
11. **Peter Thompson, Unitec NZ**
 - not opposed to the technology where a demonstrable social benefit exists (such as medical advances, reduction in fertilisers, pesticides and herbicides, or nutritional enhancement of food), however this application does not meet these criteria and therefore should be opposed.
 - it is likely that glyphosate causes some cumulative physiological harm
 - Monsanto applications should be viewed with the utmost suspicion.
12. **Shushila Ajani**
 - opposed to the Application for numerous reasons including concerns about health, the environment, the economy, and profits for large corporations such as Monsanto.
13. **Pam Parsons**
 - strongly opposed to the Application because of unresolved safety issues.
14. **Me Aroha Waiheke Foundation**
 - express environmental concerns with GE crops eg. rice.
 - new viruses could emerge with the use of viral genes in GE plants.
15. **Colin Day**
 - opposed to all GE applications in order to keep NZ GE-free for protection of the economy.

16-134. Doreen M. Adams; Erwin Alber; Chris and Maria Aulman; Dee Austring; Pauline and David Bailey; Annmarie Banchy; Rosemary Bartle and family; Ruth Begg; Taleb Bench-Kanjou; Graham Bennett; Paul Bradley; Lisa Bridson; Kent Briggs; Paul Brimecombe; Fiona Brodie; Tony and Kalani Bruce; Berthine Bruinsma;

J. Carapiet; M Ni Chonaola; JR Collins; Peter and Marion Corby; Dayahn Cornelius; Mona-Lynn Courteau; Harold Curry; Victoria Davis; Amy Donovan; Charles Drace; K Du Pont; Helen Eggers; Judy Erasmuson; Jodene Fabian; Sue Ferrabee; Shari French; Lillian Fougere; Ann Fullerton; David Graham; Jan Gerritsen; William Green; David Grove; Hans and Rosemary Grueber (Green Society Inc); Elizabeth Harrington; Annette Hart; Julie Hartley; Colin Hewens; Maureen Howard; Rita Hunt; Phil Hurdle; Helen Varley Jamieson; Quentin Jamieson; Hilary Jones; K.A.G.E. (Simon); Rosie Kaplan; Andy Kirk; Jane Landman; Anne Larsen; Marlene Laureys; Shaun Lee; Raylene Lodge (and family); Magnolia Productions (Morag Brownlie); Lesley MacDonald; Rose Mackinnon; Dugald MacTavish; Corrine and D. C. McBride; Mary McCammon; Mike McCree; Emily McDowell; Wendy McGuinness; Shona McKee; Carol McLean; Mario McMillan; Mandy McMullin; G. Mabbs; Jan Mabey; Vicki Martin; Daniel Meares; Jo Metz; Robert Mignault; Ian Murphy; Wim Oosterhoff; Nicky Owers; Don Paterson; Anthony Peacocke; Jane Pearce; Jennifer Pearson; Liz Peters; Richard and Tracy Pettinger; Planetary Network (Mr Royal); Hilary Philips (Jews for GE-Free Food); Trish Puharich; Stephen Richardson; Joan Roesch; Ian Roger; Tara Ross-Watt; Marg Sellers; Amanda Semb; Mark Sidebotham; Neil Sloan; Anne Smith; Gillian Somerville; Hanne Sorensen; Sam Storey; Campbell Sturrock; Ali Symmons and family; Miles Thomas; Colin Thomson; Max Tobin; Ed Tye; Clare Tyler; Kevin Tutt; L.K. Vasbenenter; Raymond Vogt; Nerine Walbran; Charles Watson; Grant Walters; Patricia Waugh; Liz Westbrooke; John and Betty Wheeler; Melanie White; Steve Williams.

- opposed to all GE food applications because of safety concerns and environmental concerns and the desire for New Zealand to be GE-Free.

Submissions from Australia

135. Food Technology Association of Victoria (David Gill)

- supports approval of MON 88017 corn.

136. Environmental Health Service, South Australia Department of Health

- the application should proceed to Draft Assessment
- the Regulatory Impact Analysis should reflect the Australian situation only and not discuss potential benefits to growers overseas.

137. Victorian Department of Human Services

- no concerns expressed in relation to the assessment of MON 88017 corn.

138. Queensland Health

- await the Draft Assessment Report before stating support or opposition
- express concerns with the cost of testing if food derived from MON 88017 corn is approved, and the Applicant should be obliged to provide methodology and reference material to assist with enforcement capabilities.

139. Australian Food and Grocery Council (AFGC)

- supports approval of MON 88017 corn, subject to the completion of a satisfactory safety assessment by FSANZ.