FOOD STANDARDS
Australia New Zealand
Te Mana Kounga Kai – Ahitereiria me Aotearoa

1 October 2008
[17-08]

FIRST REVIEW REPORT

APPLICATION A595

FOOD DERIVED FROM INSECT-PROTECTED CORN LINE MON 89034

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

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1. Introduction

On 30 July 2008, the Australia and New Zealand Food Regulation Ministerial Council (Ministerial Council) requested a First Review of Application A595, which seeks approval of food derived from a genetically modified (GM) corn – namely, insect-protected corn line MON 89034. Approval of this Application involves a variation to Standard 1.5.2 – Food produced using Gene Technology, of the *Australia New Zealand Food Standards Code* (the Code).

Following a request for a formal review, FSANZ has three months to complete a response. In this instance, FSANZ was required to review the decision by 30 October 2008.

2. Objectives of Review

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

3. Grounds for the Review requested by the Ministerial Council

A First Review of FSANZ's decision to approve Application A595 was sought on the grounds that the proposed amendment to Standard 1.5.2, to permit the sale and use of food derived from insect-protected corn line MON 89034:

(i) does not protect public health and safety;

(ii) does not provide adequate information to enable informed choice; and

(iii) is difficult to enforce or comply with, in both practical or resource terms.

3.1 Protection of public health and safety

A number of reasons has been put forward in asserting that the decision to approve food derived from corn line MON 89034 does not protect public health and safety.

Firstly, FSANZ is asked to clarify what is known about potential health implications of work establishing proof of principle for persistence and uptake of foreign DNA in and across the gastrointestinal (GI) tract of mammals. The rationale for requesting a First Review of corn line MON 89034 on these grounds is identical to that used for the First Review of Applications A592 (glyphosate-tolerant soybean line MON89788) and A589 (glufosinate ammonium-tolerant rice line LLRICE62).

Secondly, an application for approval of corn line MON 89034 to the European Food Safety Authority (EFSA) includes compositional studies on MON 89034 conducted in Argentina in 2004/2005, whereas the Final Assessment Report for Application A595 produced by FSANZ, refers to compositional data from trials conducted in 2004 in the United States. FSANZ is asked to explain why it did not request and use the additional compositional studies. The same issue was also raised in the First Review request for Application A589.

Thirdly, clarification is requested as to whether MON 89034 and control samples used in the compositional analysis were pure, as contamination of non-GM control samples with GM material would mask differences and reduce the confidence that can be placed in a conclusion of equivalence. The concern arises as a previous safety assessment, for glyphosate-tolerant soybean MON 89788 (A592) acknowledged contamination of one of the non-GM control samples with GM material (≤ 3.05%) and the Review request states that such contamination may not be unusual. It is stated that, for MON 89034, FSANZ should determine whether purity was adequately assessed, the outcome of that assessment, and if contamination occurred, clarify the policy it applies when evaluating compositional analysis results, including whether a contamination tolerance has been set.

Fourthly, the experimental design of the rat feeding study summarised in the Final Assessment Report is questioned. The First Review request asserts that the proponent's feeding study, as described in the Final Assessment Report, cannot be considered as evidence of the safety of MON 89034.

Finally, it is claimed that independent safety testing should be undertaken by FSANZ and is imperative to ensure that the FSANZ safety assessment is an objective, transparent process that can provide consumers with confidence in the safety of foods. This same issue was also raised in the First Review request for Application A589.

3.2 Provision of adequate information to enable informed choice

The First Review request states that current GM labelling regulations are inadequate in providing consumers with sufficient information to make informed purchase decisions.

3.3 Enforcement and compliance

It is claimed that current enforcement and monitoring of GM food regulation is not being adequately undertaken due to a lack of resources. It is believed that the lack of enforcement activity therefore warrants a cautious approach to approving GM applications. This same issue was also raised in the First Review request for Application A589.

4. Background

FSANZ received an Application from Monsanto Australia Ltd (the Applicant) on 19 December 2006. The Applicant requested a variation to the Code, specifically to Standard 1.5.2, to permit the sale and use of food derived from a genetically modified (GM) variety of corn, MON 89034. To be approved for food use in Australia and New Zealand under this Standard, GM foods undergo a pre-market safety assessment, which is conducted by FSANZ.

MON 89034 corn has been genetically modified to be protected against feeding damage caused by the larvae of certain insect pest species. Protection is achieved through the expression in the plant of insecticidal proteins derived from *Bacillus thuringiensis*, a common soil bacterium.

Corn line MON 89034 is intended to be grown in North America. However, once commercialised, corn products imported into Australia and New Zealand could contain ingredients derived from MON 89034 corn.

Approval is therefore necessary before these products may enter Australian and New Zealand markets. Corn line MON 89034 has already received approval in the United States (Food, Feed and Environment), Canada (Feed and Environment) and Japan (Food, Feed and Environment).

Prior to approval, FSANZ completed a comprehensive safety assessment of food derived from insect-protected corn line MON 89034. The assessment included consideration of: (i) the genetic modification to the plant; (ii) the potential toxicity and allergenicity of the novel proteins; and (iii) the composition of MON 89034 corn compared with that of conventional corn varieties. No public health and safety concerns were identified as a result of the safety assessment.

5. Conclusions from the Final Assessment Report

The Executive Summary and the reasons for the decision, which were approved by the FSANZ Board in May 2008, are provided in this Report at **Attachment 2**.

The Board agreed to the recommendation at Final Assessment to approve food from corn line MON 89034 in view of the findings of the safety assessment that food derived from this line is as safe and wholesome as food derived from other commercial corn varieties.

6. Issues addressed in First Review

6.1 Ingestion of recombinant DNA in food

The issue of persistence and uptake of recombinant DNA, when ingested, is a general issue that has been the subject of extensive consideration and publication for more than 15 years. Based on these deliberations and prolonged scientific discourse, the consensus is that as DNA from all living organisms is structurally similar, the presence of recombinant DNA in food products, in itself, poses no additional health risk to consumers (WHO 1991, WHO 1993, Karenlampi 1996, Jonas *et al* 2001, Gaye & Gillespie 2005, Flachowsky et al 2007, EFSA 2007)[1].

While the issue continues to be an active area of research and publication, FSANZ does not regard this as an issue that requires specific and explicit consideration for each and every GM food assessment. FSANZ continues to monitor the scientific literature for studies relevant to the safety assessment of GM foods and is fully cognisant of the literature relating to the uptake and persistence of recombinant DNA when ingested as part of GM food. A detailed response on this issue prepared for the review of Applications A592, is presented at **Attachment 3** to this Report.

6.2 Additional compositional studies were available

The studies submitted to FSANZ with Application A595 included a compositional analysis of MON 89034 corn in comparison to conventional corn lines under typical cultivation conditions. These studies are designed to identify any compositional differences in MON 89034 corn as a result of the modification, and to assess its nutritional adequacy.

[1]Full citations are listed in Attachment 3.

Compositional analyses were done on forage and grain samples collected from MON 89034, a conventional control line with the same genetic background as MON 89034, and 15 commercial corn hybrids grown under field conditions. Field trials were conducted in the United States of America (USA) in 2004 at five replicated sites. The field sites were located in regions of the USA that are suitable for the growth of corn and which are representative of commercial corn production. Seed was planted in a randomised complete block design with three replicates per block of each MON 89034, control and reference line. All the corn lines at each of the field sites were grown under normal field conditions for their respective geographic regions. The components analysed were protein, fat, carbohydrate, amino acids, fatty acids, vitamins, minerals, and the anti-nutrient phytic acid, in accordance with OECD guidance[2]. Methods of analysis were based on internationally recognised procedures (e.g. AOAC International methods) or other published methods.

The study submitted by the Applicant conformed to the requirements of the FSANZ *Application Handbook*[3] and guidance document on the safety assessment of genetically modified foods[4].

No differences of biological significance were observed between MON 89034 corn and its conventional counterpart. Some minor differences in key nutrients were noted, however the levels observed were within the range of values measured for commercial corn hybrids and other conventional corn varieties. Food from MON 89034 corn is therefore considered to be compositionally equivalent to food from conventional corn varieties.

The Applicant has advised that the results of the compositional analyses conducted in the USA were submitted to global regulatory authorities. As MON 89034 is intended primarily for cultivation in the USA, the results of compositional studies conducted in the USA are considered the most relevant. The data requirements for assessment in Europe differ, in that Applicants are required to submit data from two growing seasons. As a consequence, the results of additional compositional studies conducted in Argentina were supplied to EFSA in 2007. The results of both studies were also published in the peer-reviewed *Journal of Agricultural and Food Chemistry* in 2008[5].

The field trials conducted in Argentina essentially reproduced the data generated in the North American studies. These field trials included five replicated sites during the 2004-2005 growing season. The replicated trials were based on a randomised complete block design with three replicates per block of each test, control and commercial corn hybrids. Corn plants were grown under normal field conditions for their respective geographic locations. Compositional analyses were done on forage and grain samples collected from MON 89034, a conventional control line with the same genetic background as MON 89034, and 15 commercial corn hybrids. The components analysed and methods of analysis used were as for the U.S. field trial.

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[2] OECD (2002). Consensus document on compositional considerations for new varieties of maize (*Zea mays*): key food and feed nutrients, anti-nutrients and secondary plant metabolites. Organisation for Economic Cooperation and Development, Paris.

[3] http://www.foodstandards.gov.au/_srcfiles/Application%20Handbook%20as%20at%205%20June%2008.pdf

[4] http://www.foodstandards.gov.au/_srcfiles/GM%20FINAL%20Sept%2007L%20_2_.pdf

[5] Drury, S.M., Reynolds, T.L., Ridley, W.P., Bogdanova, N., Riordan, S., Nemeth, M.A., Sorbet, R., Trujillo, W.A. and Breeze, M.L. (2008) Composition of Forage and Grain from Second-Generation Insect-Protected Corn MON 89034 is Equivalent to that of Conventional Corn (*Zea mays* L.). *J. Agric. Food Chem*. **56**:4623-4630.

The published results of the field trials conducted in Argentina confirm that the composition of MON 89034 corn is equivalent to that of conventional corn hybrids. While a few statistically significant differences between MON 89034 corn and the conventional counterpart were reported, these likely reflect the natural variability of the individual components since the mean levels of the specific nutrients in question are within the tolerance intervals for commercial corn hybrids, as well as the ranges reported in the scientific literature and the ILSI Crop Composition Database[6].

The conclusion from both studies is that corn line MON 89034 is compositionally equivalent to conventional corn. While additional evidence is always welcomed, the original studies provided with this Application fulfilled compulsory data requirements and adequately demonstrated that MON 89034 corn is equivalent in composition to its conventional counterpart. In determining absolute data requirements, as distinct from those that FSANZ regards as non-essential, it is important to distinguish information that merely corroborates the core scientific evidence.

6.3 Purity of samples used for compositional analyses

The study report provided by the Applicant on the compositional analyses of corn from MON 89034 stated that 'the identities of the test, control and reference substances were verified by the Study Director prior to their use in the study by confirming the chain-of-custody documentation supplied with the samples collected from the field. The grain samples from the test, control and reference substances were further characterized by an event-specific PCR analysis of DNA extracted from grain to confirm the presence or absence of each event. The presence or absence of MON 89034 in respective samples of the grain from the test and control substances were confirmed.'

The Applicant has also provided additional information on the verification of purity of the test material used for compositional analyses. Event-specific testing of the parent generation used to produce the test material indicated a purity of greater than 98%. Since purity of the parent material was already established, the testing strategy for material used in the compositional analyses was to evaluate for adventitious presence of non-MON 89034 events that may complicate interpretation of the results. Thus, confidence in the identity of the test material is based on 'a combination of event specific purity testing prior to planting in this study, completed chain of custody documentation during the study and the event specific pooled testing scheme performed after harvest.'

The quality control information provided by the Applicant indicates that the methods used to prepare and identify the materials used in the compositional analyses complied with Good Laboratory Practice (GLP). FSANZ is therefore satisfied with the overall conduct of the compositional studies, including determination of the purity of the samples used in the analyses, and considers that the conclusions of the study are scientifically valid.

[6] International Life Science Institute Crop Composition Database, version 2.0 http://www.cropcomposition.org

6.4 The use and design of animal feeding studies

FSANZ's safety assessment of food derived from insect-protected corn line MON 89034, included consideration of: (i) the genetic modification to the plant; (ii) the potential toxicity and allergenicity of the novel proteins; and (iii) the composition of MON 89034 corn compared with that of conventional corn varieties. No public health and safety concerns were identified as a result of the safety assessment. The safety assessment did not rely on the results of a rat feeding study submitted with the Application. While FSANZ does not routinely require animal feeding studies to be undertaken, where such studies already exist, Applicants are expected to provide these to FSANZ to evaluate as additional supporting information.

Despite continuing claims that animal studies with GM foods can be designed without inherent flaws, there has been no consensus of expert scientific opinion in relation to appropriate methodology. For example, for whole foods it is difficult to feed experimental animals with a range of doses, including excessive doses of 100-fold or more than the concentration in the expected human diet, as is the norm in traditional toxicological testing of single chemicals. At high levels of dietary incorporation, nutritional imbalances and deficiencies are likely to occur. With increasing levels of dietary incorporation, an increasing number of observed effects are not toxicologically relevant and are a consequence of high doses leading to secondary effects, for instance due to nutrient imbalances or metabolic overload.

The First Review request cites a recent publication[7] of an immunotoxicological study that reports an antibody response in control rats housed alongside rats fed GM material and raises concerns about immunological effects possibly induced by inhaled food particles. The authors raise the question whether separate housing for control and test animals would provide greater assurance that any immunological responses due to the test diet could be detected, particularly when performing feeding studies with dry non-pelleted diets.

The publication is the work of a European research project, known as the SAFOTEST project, which had the objective of improving the sensitivity and specificity of GM food safety assessment. This project was particularly focussed on improving the standard OECD 90-day rodent study and adapting it to the study of GM foods. The 90-day feeding study in SAFOTEST uses one control group and one dose group, both receiving the highest nutritionally tolerable intake level of the food.

This example highlights the difficulties in designing and conducting animal feeding studies that overcome inherent experimental problems. In recommending that test and control animals be housed in separate rooms, the authors potentially introduce another set of variables that would need to be considered in interpreting their results.

While deviations from basic procedures involved in conducting animal experiments or improperly designed experiments can lead to flawed interpretations of the results, these problems should not be generalised.

[7] Kroghsbo, S., Madsen, C., Poulsen, M., Schroder, M., Kvist, P.H., Taylor, M., Gatehouse, A., Shu, Q. and Knudsen, I. (2008) Immunotoxicological studies of genetically modified rice expressing PHA-E lectin or Bt toxin in Wistar rats. Toxicology 245:24-34.

In this case, the feeding study submitted by the Applicant as supporting information in the assessment of A595 was based on OECD Guidelines[8]. This is one of a series of OECD guidelines that are internationally recognised as the standard for testing of single chemicals. The protocol was adapted for the study of a whole food.

The First Review request states that the feeding study supplied by the Applicant cannot be considered as evidence of the safety of MON 89034. While FSANZ acknowledges that there are numerous challenges in establishing an experimental protocol for animal feeding studies with whole foods, the safety assessment did not rely on this study in reaching the conclusion that MON 89034 corn is as safe as its conventional counterpart.

Technical limitations of whole food animal feeding studies are one of the reasons that FSANZ does not rely on these types of studies in its safety assessment of GM foods. FSANZ has addressed the issue of animal feeding studies previously and has posted further information on the website [9].

6.5 FSANZ has not conducted independent safety testing

This issue has been raised previously and the following text is extracted from a 'frequently asked questions' page on the FSANZ website[10].

> The responsibility for demonstrating the safety of any new food product on the market lies with the developer of that product. This is also the case for new chemicals and drugs. When an applicant seeks approval for a new GM food, they must provide FSANZ with the evidence that supports the safety of the product. It is a requirement that this data be generated according to quality assurance guidelines that are based on internationally accepted protocols (i.e. validated methodology and procedures that are consistent with Good Laboratory Practice (GLP)) and stand up to external scrutiny (i.e. independent audits and documentation trails). To achieve this, the applicant submits to FSANZ a comprehensive dossier of quality-assured raw experimental data for each GM food. This enables FSANZ to independently assess the data and reach a conclusion about the safety of the food.
>
> FSANZ also complements the data package provided by the applicant with information from the scientific literature, other applications, other government agencies and the public.
>
> Paper reviews are a standard scientific method of evaluation used by regulators around the world, to evaluate the health and safety of a variety of products including food, drugs and agricultural and veterinary chemicals. The methods and approach used by FSANZ are wholly consistent with international guidelines developed according to scientific advice provided by the WHO, FAO, and OECD.
>
> Companies involved in the development of GM foods spend millions of dollars rigorously testing their products according to these requirements, which include detailed documentation of testing. Thorough analysis is conducted of the data and of the protocol used to ensure the validity of results. If FSANZ determines that the data are not sufficient, additional information and testing may be required. FSANZ may also supplement the information provided by the Applicant with any published data in Australia and New Zealand or internationally that is relevant to the product in question.

[8] OECD (1998) OECD Guidelines for Testing of Chemicals No. 408, Repeated dose 90-day oral toxicity study in rodents. Paris, France.
[9] http://www.foodstandards.gov.au/foodmatters/gmfoods/frequentlyaskedquest3862.cfm
[10] http://www.foodstandards.gov.au/foodmatters/gmfoods/frequentlyaskedquest3862.cfm

6.6 Current GM labelling regulations are inadequate

The First Review request states that current GM labelling regulations are inadequate in providing consumers with sufficient information to make informed purchase decisions.

Health Ministers comprising the former Australia New Zealand Food Standards Council (ANZFSC) resolved in July 2000 to require labelling of GM foods with the words 'genetically modified' where novel DNA and/or protein from an approved GM variety is present in the final food, or where the GM food has altered characteristics. The Ministers resolved that highly refined food, such as oils, sugars and starches that have undergone refining processes that have the effect of removing DNA and/or protein, would be exempt from these requirements. The labelling provisions of Division 2 of Standard 1.5.2 came into effect in December 2001. At that time, Ministers acknowledged that these broader labelling requirements were primarily to satisfy consumer information issues and were not based on any safety concerns.

GM labelling was reviewed by FSANZ in 2003 in the *Review of Labelling of Genetically Modified (GM) Foods* (available from the FSANZ website at http://www.foodstandards.gov.au/newsroom/publications/gmlabellingreviewrep2460.cfm). The Review found that the labelling requirements for GM foods prescribed in Standard 1.5.2 were rigorous and remain among the most comprehensive, both in scope and breadth of capture, of any country in the world.

As the safety of a GM food is thoroughly assessed prior to approval, the purpose of labelling GM foods is to provide information to consumers, allowing them to purchase or avoid such foods depending on their own views and beliefs. The labelling requirements represent a balance between the desire to provide information to consumers and the ability of government agencies to enforce such requirements.

6.7 Current enforcement and monitoring of GM food regulation is inadequate

It is claimed that current enforcement and monitoring of GM food regulation is not being adequately undertaken due to a lack of resources. It is believed that the lack of enforcement activity therefore warrants a cautious approach to approving GM applications.

The NSW Food Authority has raised the issue of developing a national GM compliance and enforcement strategy with the Implementation Sub-Committee (ISC)[11] and hosted a workshop on the issue in July 2008.

Potential options for developing a national strategy discussed at the workshop have been circulated to the workshop participants and will be further discussed at ISC in November 2008. FSANZ supports the initiative of the NSW Food Authority in raising this issue at a national level, in particular the proposal that the ISC develop a national compliance and monitoring strategy.

FSANZ already adopts a cautious approach to approving GM applications, and will not approve a GM food for sale if there is any evidence of any public health and safety concerns.

[11] ISC comprises heads of the appropriate Australian (Commonwealth and State/Territory) and New Zealand inspection and enforcement agencies and is responsible, among other things, for overseeing the development and implementation of a consistent approach across jurisdictions to enforcing food regulation and standards.

Therefore, regardless of enforcement and monitoring activities, satisfactory completion of a safety assessment and approval of a GM food ensures that GM foods that are likely to be present in the Australian and New Zealand food supply are safe for human consumption.

7. Review Options

There are three options proposed for consideration under this Review:

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

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

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

8. Decision

FSANZ has considered the issues raised by the Ministerial Council in relation to Application A595 – Food derived from Insect-protected Corn Line MON 89034.

The First Review concludes that the preferred review option is Option 1. FSANZ has decided to re-affirm the variation to Standard 1.5.2 to permit the sale of food derived from insect-protected corn line MON 89034, as detailed in **Attachment 1**.

Decision

FSANZ re-affirms the variation to Standard 1.5.2 of the Code to permit the sale of food derived from insect-protected corn line MON 89034.

9. Implementation and review

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

Attachments

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

Attachment 1

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

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

To commence: on gazettal

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

Food derived from insect-protected corn line MON 89034	

Attachment 2

Executive Summary and Reasons for Decision from the Final Assessment Report

Executive Summary

Food Standards Australia New Zealand (FSANZ) received a paid Application from Monsanto Australia Ltd (the Applicant) on 19 December 2006. The Applicant has requested a variation to the *Australia New Zealand Food Standards Code* (the Code), specifically to Standard 1.5.2 – Food produced using Gene Technology, to permit the sale and use of food derived from a new genetically modified (GM) variety of corn, MON 89034. Standard 1.5.2 prohibits a food produced using gene technology from being sold or used as an ingredient or component of any food unless it is listed in the Table to clause 2 of that Standard.

MON 89034 corn has been genetically modified to be protected against feeding damage caused by the larvae of certain insect pest species. Protection is achieved through the expression in the plant of insecticidal proteins derived from *Bacillus thuringiensis*, a common soil bacterium.

Corn line MON 89034 is intended to be grown in North America. However, once commercialised, corn products imported into Australia and New Zealand could contain ingredients derived from MON 89034 corn. Approval is therefore necessary before these products may enter Australian and New Zealand markets.

Safety Assessment

FSANZ has completed a comprehensive safety assessment of food derived from insect-protected corn line MON 89034, as required under Standard 1.5.2. The assessment included consideration of (i) the genetic modification to the plant; (ii) the potential toxicity and allergenicity of the novel proteins; and (iii) the composition of MON 89034 corn compared with that of conventional corn varieties.

No public health and safety concerns were identified as a result of the safety assessment. On the basis of the available evidence, including detailed studies provided by the Applicant, food derived from insect-protected corn line MON 89034 is considered as safe and wholesome as food derived from other commercial corn varieties.

Labelling

If approved, food derived from insect-protected corn line MON 89034 will be required to be labelled as genetically modified if novel DNA and/or novel protein is present in the final food. Studies conducted by the Applicant show that the novel proteins are present at low levels in the grain.

Labelling addresses the requirement of section 18(1)(b) of the Act, namely the 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 insect-protected corn line MON 89034 based on the conclusions of the safety assessment.

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

Purpose

The Applicant seeks amendment to Standard 1.5.2 – Food produced using Gene Technology, to include food derived from insect-protected corn line MON 89034 in the Table to clause 2.

Decision

Vary Standard 1.5.2 – Food produced using Gene Technology, to include food derived from insect-protected corn line MON 89034 in the Table to clause 2.

Reasons for Decision

A variation to the Code approving food derived from insect-protected corn line MON 89034 in Australia and New Zealand is approved on the basis of the available scientific evidence, for the following reasons:

- the safety assessment did not identify any public health and safety concerns associated with the genetic modification used to produce insect-protected corn line MON 89034;
- food derived from insect-protected corn line MON 89034 is equivalent to food from the conventional counterpart and other commercially available corn varieties in terms of its safety for human consumption and nutritional adequacy;
- labelling of certain food commodities derived from insect-protected corn line MON 89034 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 preferred option is option 2, an amendment to the Code.

Consultation

The Initial Assessment was advertised for public comment between 21 March and 2 May 2007. A total of fourteen submissions were received during this period. The Draft Assessment was advertised for public comment between 12 December 2007 and 6 February 2008. A total of thirteen submissions were received. A summary of these is provided in **Attachment 3** to this Report.

Attachment 3

The safety of recombinant DNA in food

1. Recombinant DNA is no different to DNA from non-GM sources

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

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

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

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

Genetic modification typically results in the introduction of one or two new genes into an organism's genome. Given the large size of plant genomes, the contribution made by recombinant DNA to the total DNA in the genome will be very small. For example, for corn, which has an average genome size of 2,292 Mb, transformed with an insert of approximately 5 kb, the inserted recombinant DNA will make up only 2 X 10,000% of the total DNA in the genome (Jonas et al 2001).

4. Nucleic acids are broken down during food processing

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

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

5. Ingested nucleic acids are extensively broken down in the digestive tract

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

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

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

In the Netherwood et al study, nineteen human volunteers (twelve with intact digestive tracts, seven with ileostomies[12]) were fed GM soy containing the *epsps* gene. The amount of recombinant DNA that survived passage through the small bowel varied between the seven ileostomists, with a maximum of 3.7% recovered from the stoma of one individual. This rate of recovery was similar to an endogenous soy gene, suggesting the recombinant DNA was digested similarly to other plant DNA. The *epsps* gene could not be detected in faeces from subjects with intact digestive tracts, suggesting that any DNA surviving digestion in the upper GI tract is readily degraded in the large intestine. The study also found evidence of pre-existing transfer of a fragment of the *epsps* gene between GM soy and a small number of micro-organisms in the small intestine of the ileostomists. The authors speculated this had occurred prior to commencement of the study. There was no evidence of the intact *epsps* gene being transferred. In subjects with intact digestive tracts, none of the endogenous bacteria in the faeces were found to contain any *epsps* gene fragments from GM soy.

In the studies reported by Schubbert et al, M13 bacteriophage DNA was fed to mice at high doses and transiently detected as fragments in various tissues including foetal tissue. The vast majority of cells identified as containing M13 DNA fragments appeared to be macrophages or other differentiated phagocytes of the immune system.

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

The purpose of such cells is to destroy foreign macromolecules. It has been suggested that the relatively high frequency of cells that contained M13 DNA is probably related to the occurrence of unmethylated CpG sequences, which would stimulate macrophages and other immune cells to phagocytose the fragments (Beever and Kemp, 2000). Unmethylated CpG sequences are characteristic of bacterial DNA but not DNA in either plants or animals, therefore M13 DNA is probably not a good model for plant-derived recombinant DNA.

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

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

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

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

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

The gene transfer mechanisms by which bacteria may acquire new genes (conjugation, transduction and transformation) are well described and a number of comprehensive reviews on these processes are available (e.g. Levy & Miller 1989). In food, transfer by all three mechanisms is believed to be possible, at least from micro-organisms consumed in food, although studies on gene transfer in the human and animal gut are limited (Jonas et al 2001).

The gut and the colon in particular are considered to be a favourable environment for such transfer because of the high density of micro-organisms; direct cell to cell contact favours conjugation, and natural transformation is also favoured because of the relatively high DNA concentration at the recipient cell surface (Paul 1992).

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

- degradation of DNA through the gastric and ileal passage makes it highly unlikely that linear DNA molecules of sufficient size will enter the colon;]

- for transformation by linear DNA the bacterial cell must be competent:

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

- DNA transferred through transduction or transformation may be susceptible to restriction by bacterial restriction endonucleases, which cleave double-stranded DNA;

- in the case of linear DNA, homology with sequences in the bacterial genome is necessary for integration to occur;

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

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

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

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

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

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

Mammalian cells have evolved with several mechanisms of defence against the uptake, integration and continued expression of foreign DNA (Doerfler 1991). In addition to the initial degradation and/or excretion of foreign DNA that occurs following ingestion and the action of cells of the immune system e.g. phagocytes, to remove foreign macromolecules, most mammalian cells produce at least one DNase with exonuclease activity, and these would be expected to degrade most exogenous DNA, should it actually survive and be taken up by the cell (Jonas et al 2001).

The nuclear membrane is also a strong barrier against the penetration of nucleic acids. Entry is tightly regulated by nuclear pores, with nuclear targeting signals required for penetration, especially in the case of cells that have finished their division and the nuclear envelope is not disrupted (Gorlick & Mattaj 1996, Guralnick et al 1996, Collas & Aelstrom 1997, Palacios et al 1997, Popov et al 1998, Zeimienovicz et al 1999, Saphire et al 2000). Should DNA succeed in penetrating the nucleus, and become integrated in the genome, the evidence indicates that any integrated foreign DNA is likely to be rendered inactive through targeted methylation (Doerfler 1991, Doerfler et al 1995, Orend et al 1995).

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

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

The study by Malatesta et al (2002) on the ultrastructure of hepatocytes from mice fed GM soybean[13], is interesting in that the authors report that the GM soy-fed mice exhibited some slight but statistically significant ultrastructural differences in hepatocyte nuclei[14] relative to controls. Cells bearing slightly more irregularly shaped nuclei were postulated to be indicative of an increased metabolic rate and the slight increase in the number of nuclear pores was apparently suggestive of increased molecular trafficking between the nucleus and cytoplasm.

The study itself is quite unusual because it undertakes an investigation at the ultrastructural level in the absence of any clear evidence of effects in the liver at either the macroscopic or light microscopic level. Typically, ultrastructural investigations are only undertaken to identify an underlying mechanism if there is clear evidence of cellular change or clinical signs. In the Malatesta et al study only 100 cells/mouse were examined. Consequently the relevance of the subtle ultrastructural morphometrical changes observed are difficult to interpret, especially in the absence of any corroborating evidence of atypical liver activity (e.g. classical markers of liver cell damage).

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

In addition, it is not clear that such effects, were they to be reproduced, would necessarily be attributable to the presence of recombinant DNA itself. The relevance of this study to the issue of persistence and uptake of recombinant DNA is therefore questionable.

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

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

9. Conclusion

The transferred DNA in MON 89034 corn does not contain any genetic elements which may significantly increase the likelihood of recombinant DNA in GM food being taken up and stably incorporated into the genome of either gut micro-organisms or human cells. Given this, FSANZ does not consider that the issue of persistence and uptake of recombinant DNA requires specific consideration in the safety assessment of food derived from insect-protected corn line MON 89034; consideration of such issues is already implicit in the molecular characterisation component of the safety assessment.

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