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

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APPLICATION A1001
FOOD DERIVED FROM INSECT-PROTECTED CORN LINE MIR162
1st REVIEW REPORT

CONTENTS

INTRODUCTION

1. The Issue

On 3 October 2008, the Australia and New Zealand Food Regulation Ministerial Council (Ministerial Council) requested a First Review of Application A1001, which seeks approval of food derived from a genetically modified (GM) corn – namely, insect-protected corn line MIR162. 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 3 January 2009.

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 A1001 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 MIR162, does not protect public health and safety.

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 MIR162 does not protect public health and safety. Firstly, the First Review request states that 'there is no confidence that the construct is safe for human consumption as there is an absence of independent long term animal feeding trials designed to measure outcomes relevant to human health.'

Secondly, FSANZ is asked to further 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. These grounds were used for the First Review of Application A592 (glyphosate-tolerant soybean line MON89788) and the First Review Report for A592 included a detailed summary of the scientific evidence relating to GI uptake of foreign DNA and the potential implications for the health of consumers. However, the First Review request for A1001 states that FSANZ's First Review Report for A592 did not provide that clarity and thus the same concerns stand for A1001.

Thirdly, clarification is requested as to whether MIR162 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 MIR162, 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, inclusion of the results of a broiler feeding study in the Safety Assessment of MIR162 is questioned. The First Review request questions whether a study designed to answer animal production questions can be used to support any conclusion regarding nutritional impact of MIR162 corn.

Further, FSANZ is requested to outline the scientific evidence utilised to support the conclusion that 'feeding GM plant material to livestock does not affect the nutritional value or safety of the meat, milk and eggs derived from those animals.'

Finally, the First Review request also notes that, to date, no country has given regulatory approval for insect-protected corn line MIR162.

4. Background

FSANZ received an Application from Syngenta Seeds Pty Ltd (the Applicant) on 10 October 2007. The Applicant requested an amendment to Standard 1.5.2 to permit the sale and use of food derived from a new genetically modified variety of corn, MIR162. 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.

MIR162 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 an insecticidal protein derived from *Bacillus thuringiensis*, a common soil bacterium.

Corn line MIR162 is intended to be grown in North America. However, once commercialised, corn products imported into Australia and New Zealand could contain ingredients derived from MIR162 corn. Approval is therefore necessary before these products may enter the Australian and New Zealand markets.

Prior to approval, FSANZ completed a comprehensive safety assessment of food derived from insect-protected corn line MIR162. 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 MIR162 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 July 2008, are provided in this Report at **Attachment 2**.

The Board agreed to the recommendation of the Approval Report to approve food from corn line MIR162 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.

ISSUES ADDRESSED IN FIRST REVIEW

6. Independent long term animal feeding trials

The First Review request states that 'there is no confidence that the construct is safe for human consumption as there is an absence of independent long term animal feeding trials designed to measure outcomes relevant to human health.' The issue of long term animal feeding studies was addressed in response to submissions received during the public consultation period for A1001, and has also been addressed in the First Review Report for Application A595 (food derived from insect-protected corn line MON 89034). FSANZ's response to this issue is reiterated below.

FSANZ's safety assessment of food derived from insect-protected corn line MIR162, 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 MIR162 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 the feeding study in rapidly-growing broiler chicks.

FSANZ considers that a scientifically-informed comparative assessment of GM foods with their conventional counterparts can generally identify any potential adverse health effects or differences requiring further evaluation. In the majority of circumstances, animal toxicity studies with whole foods are not likely to contribute any further useful information to the safety assessment and are therefore not warranted. As a consequence, FSANZ does not require that animal feeding studies with whole GM foods be undertaken on a routine basis.

FSANZ acknowledges there may be future GM applications, particularly for foods with intentional modifications to composition, where the results of animal toxicity studies may be informative. FSANZ therefore continues to assess the need for animal studies on a case-by-case basis, taking into account the nature of the genetic modification and the results of the comparative assessment. While FSANZ does not routinely require animal toxicity studies to be undertaken, where such studies already exist, Applicants are expected to provide these to FSANZ to evaluate as additional supporting information.

As part of a continual review of FSANZ's scientific approach to the safety assessment of GM foods, FSANZ convened an expert panel in June 2007 to develop guidance and recommendations on the role animal feeding studies can play in the safety assessment of GM foods. The expert panel recommended that FSANZ should continue with its case-by-case assessment of GM foods on the basis of best available science.

The panel noted that whole-food animal feeding studies may be informative in some limited circumstances, but concurred with FSANZ that any potential adverse health effects can generally be identified by a scientifically informed comparative assessment of the GM food against its conventional counterpart. The report from the expert panel is available from www.foodstandards.gov.au/_srcfiles/Workshop%20Report%20FINAL.pdf

The GMO Panel of the European Food Safety Authority recently adopted a similar approach and recommends 'In cases where molecular, compositional, phenotypic, agronomic and other analyses have demonstrated equivalence between the GM plant derived food and feed and their conventional counterpart, except for the inserted trait(s), and results of these analyses do not indicate the occurrence of unintended effects, the performance of animal feeding trials with rodents or with target animal species adds little if anything to the overall safety assessment, and is not recommended'[1].

Further information is available on the FSANZ website [2].

7. Ingestion of recombinant DNA in food

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 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)[3].

This issue was raised in the First Review requests for Applications A592 (glyphosate-tolerant soybean line MON89788), A589 (glufosinate ammonium-tolerant rice line LLRICE62) and A595 (insect-protected corn line MON 89034). In response FSANZ prepared a detailed review of the scientific evidence relating to GI uptake of foreign DNA and the potential implications for the health of consumers. This response is presented at **Attachment 3** to this report.

However, the First Review request for Application A1001 states that FSANZ's First Review Report for A592 did not provide sufficient clarity and thus the same concerns stand for A1001.

FSANZ believes that the First Review Report for Application A592 provides a detailed review of the scientific evidence for persistence and uptake of foreign DNA across the GI tract and comprehensively discusses the potential health implications of this work. Thus, FSANZ believes that it has adequately addressed this issue.

In relation to this Application, the transferred DNA in MIR162 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 MIR162; consideration of such issues is already implicit in the molecular characterisation component of the safety assessment.

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[1] EFSA (2008) Safety and nutritional assessment of GM plants and derived food and feed: The role of animal feeding trials Report of the EFSA GMO Panel Working Group on Animal Feeding Trials. *Food and Chemical Toxicology* 46 (2008) S2–S70 http://www.efsa.europa.eu/cs/BlobServer/Non_Scientific_Document/gmo_report_feedingtrials.pdf?ssbinary=true

[2] http://www.foodstandards.gov.au/foodmatters/gmfoods/frequentlyaskedquest3862.cfm

[3] Full citations are listed in Attachment 3.

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.

8. Purity of samples used for compositional analyses

The First Review request seeks clarification as to whether the samples of GM and control corn used for compositional analysis were pure.

The Applicant has advised FSANZ that the samples used for the compositional analysis of MIR162 corn were tested for purity. Testing consisted of one hundred kernel samples from each lot (MIR162 and control) analyzed by Taqman PCR using a panel of assays to check for the presence of approved GM lines as well as lines still under development by the Applicant. No contamination was detected in these samples, which provides 95% confidence that the lot impurity is 3% or less.

FSANZ is 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.

9. The inclusion of livestock feeding studies in nutritional assessment

The First Review request questions the validity of including the results of a broiler feeding study in the safety assessment report for MIR162 corn. The request asserts that such studies should only be included where they have clear relevance to food safety, and that it is debatable whether a study designed to answer animal production questions bears such relevance. The request states that including results of such studies as supporting information in a safety assessment is misleading.

FSANZ agrees that studies designed to evaluate survival, growth, feed conversion and carcass characteristics of target livestock species have limited value in the safety assessment of most GM foods.

In cases where a GM food has been shown to be compositionally equivalent to conventional varieties, the evidence to date indicates that feeding studies using target livestock species will add little to the safety assessment and generally are not warranted[4].

In the case of MIR162 corn, the Applicant submitted a feeding study comparing the nutritional performance of MIR162 corn with a non-transgenic near-isogenic control corn and a commercially available conventional corn. This study was evaluated by FSANZ as additional supporting information of the nutritional equivalence of MIR162 corn to conventional corn varieties, but was not considered essential to the safety assessment of food derived from MIR162 corn. The detailed compositional studies are considered adequate to establish the nutritional adequacy of food derived from insect-protected MIR162 corn. However, evaluation of the feeding study in rapidly-growing broiler chicks confirmed that MIR162 corn is equivalent to its conventional counterpart and commercial corn in its ability to support typical growth and well-being.

[4] OECD (2003) *Considerations for the safety assessment of animal feedstuffs derived from genetically modified plants*. Series on the Safety of Novel Foods and Feeds, No. 9. Organisation for Economic Cooperation and Development, Paris.

While the broiler feeding study supplied by the Applicant was included in the safety assessment of MIR162 corn as additional supporting information, the safety assessment report clearly states that FSANZ considers such studies to be of limited value. However, FSANZ is also mindful of the concerns of some consumers regarding the perceived need for, or at least reassurance provided by, such studies, and that exclusion of such studies from consideration by FSANZ may raise concerns amongst some consumers. Therefore, FSANZ policy is to include an evaluation of such studies, where they are provided, while bearing in mind the potential limitations of such studies.

10. The safety of food derived from animals fed GM feed

The First Review request notes that FSANZ does not have the legislative mandate to assess the safety of food derived from animals fed GM feed, but seeks elaboration of the scientific evidence supporting the conclusion stated in the A1001 Approval Report that 'the scientific evidence published so far indicates that feeding GM plant material to livestock does not affect the nutritional value or safety of the meat, milk and eggs derived from those animals.'

The following response to this issue is extracted from the FSANZ Guidance Document on the Safety Assessment of Genetically Modified Foods[5].

> Many animal feeds are derived from the same GM commodities that are used for human consumption and concerns are occasionally expressed that this practice may pose an indirect risk to humans through consumption of the meat, milk and eggs derived from such animals.
>
> The OECD has produced a paper as part of their series on the safety of novel foods and feeds entitled 'Considerations for the safety assessment of animal feedstuffs derived from genetically modified plants'[6]. This paper specifically examined potential hazards to humans from the presence of novel DNA and protein in animal feedstuffs. They noted that both DNA and protein are extensively digested when consumed by animals. It was concluded that while fragments of plant genomic (non-GM) DNA have been detected in animal food products such as milk, there is no basis to suppose that recombinant DNA poses hazards any different to other sources of DNA and the possibility of incorporation of functionally intact DNA or protein derived from GM feeds into animal products is extremely remote.
>
> In addition to the above report, an overview was conducted of regulatory assessments of GM foods and to summarise empirical data generated for assessing the safety of meat, milk and eggs derived from animals fed GM crops that express agronomic input traits (e.g. herbicide tolerance)[7]. It was concluded that there are no effects from feeding GM plant material to livestock and poultry on the nutritional value or safety of the meat, milk and eggs derived from those animals. Moreover, because most components of feeds are broken down into smaller components during digestion by the animal, proteins and DNA derived from the GM plants cannot be detected in milk, meat or eggs.

[5] http://www.foodstandards.gov.au/_srcfiles/GM%20FINAL%20Sept%2007L%20_2_.pdf
[6] OECD (2003) *Considerations for the safety assessment of animal feedstuffs derived from genetically modified plants*. Series on the Safety of Novel Foods and Feeds, No. 9. Organisation for Economic Cooperation and Development, Paris..
[7] CAST (2006). *Safety of Meat, Milk, and Eggs from Animals Fed Crops Derived from Modern Biotechnology*. Issue Paper 34. Council for Agricultural Science and Technology, Ames, Iowa.

The European Food Safety Authority has also recently prepared a literature survey on the fate of recombinant DNA or proteins in meat, milk and eggs from animals fed with GM feed[8]. On the basis of this literature survey, EFSA concluded that: (i) DNA and protein are common constituents of foods and feeds and such substances are rapidly degraded into small fragments upon digestion by animals and humans; and (ii) a large number of experimental studies with livestock have shown that recombinant DNA fragments or proteins derived from GM plants have not been detected in tissues, fluids or edible products of farm animals or other livestock.

In addition, where GM crops have already been assessed by FSANZ for their safety as human food, it seems highly unlikely that a GM crop, found as safe for human food, would pose an indirect risk to humans via food products derived from animals (e.g. meat, milk, eggs) that had eaten the GM crop, when no direct risk to humans had been identified in the human food safety assessment.

11. MIR162 corn has not been approved anywhere in the world

The First Review request notes that, to date, no country has given regulatory approval for insect-protected corn line MIR162.

As is often the case for new foods and food ingredients, submissions for regulatory approval of MIR162 corn were made to a number of countries at similar times, such that the assessment processes are being undertaken in parallel.

Submissions for food, feed and environmental approvals of MIR162 corn have been made to the appropriate US agencies (Environmental Protection Agency, Food and Drug Administration, Department of Agriculture) and Canadian agencies (Health Canada and the Canadian Food Inspection Agency). Regulatory submissions for food, feed and environmental approvals have also been made in Colombia, Brazil and Argentina. Regulatory submissions for import approvals for MIR162 corn have been made in key export markets, for example, Japan, Taiwan, Korea and the Philippines. The Applicant has advised that MIR162 regulatory approvals are anticipated in 2009.

As MIR162 corn is intended for cultivation in North America, it will not be grown commercially, nor be present in the food supply, prior to receiving appropriate regulatory approvals in the United States and Canada.

CONCLUSION

12. Review Options

There are three options proposed for consideration under this Review:

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

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

[8] EFSA (2007). Statement on the fate of recombinant DNA or proteins in meat, milk and eggs from animals fed with GM feed, http://www.efsa.europa.eu/cs/BlobServer/Statement/gmo_EFSA_statement_DNA_proteins_gastroint.pdf?ssbinary=true

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

13. Decision

FSANZ has considered the issues raised by the Ministerial Council in relation to Application A1001 – Food derived from insect-protected corn line MIR162.

The First Review concludes that the preferred review option is Option 1. FSANZ has decided to re-affirm the variation to Standard 1.5.2 of the Code to permit the sale of food derived from insect-protected corn line MIR162, 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 MIR162.

14. 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*

Subsection 87(8) of the FSANZ Act provides that standards or variations to standards are legislative instruments, but 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 MIR162	

Attachment 2

Executive Summary and Reasons for Decision from the Approval Report

Executive Summary

Purpose

Food Standards Australia New Zealand (FSANZ) received an Application from Syngenta Seeds Pty Ltd (the Applicant) on 10 October 2007. The Applicant has requested an amendment 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, MIR162. 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.

MIR162 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 an insecticidal protein derived from *Bacillus thuringiensis*, a common soil bacterium.

Corn line MIR162 is intended to be grown in North America. However, once commercialised, corn products imported into Australia and New Zealand could contain ingredients derived from MIR162 corn. Approval is therefore necessary before these products may enter the Australian and New Zealand markets.

The Application is being assessed under the General Procedure and is at the Approval stage.

Safety Assessment

FSANZ has completed a comprehensive safety assessment of food derived from insect-protected corn line MIR162, 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 MIR162 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 MIR162 is considered as safe and wholesome as food derived from other commercial corn varieties.

Labelling

If approved, food derived from insect-protected corn line MIR162 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 in the grain.

Labelling addresses the objective set out in section 18(1)(b) of the *Food Standards Australia New Zealand Act 1991* (FSANZ Act); 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 MIR162 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 food derived from insect-protected corn line MIR162 is the preferred option as the potential benefits to all sectors outweigh the costs associated with the approval.

Assessing the Application

In assessing the Application and the subsequent variation to Standard 1.5.2, FSANZ has had regard to the following matters as prescribed in section 29 of the FSANZ Act:

- Whether costs that would arise from a variation to Standard 1.5.2 approving food derived from insect-protected corn line MIR162 outweigh the direct and indirect benefits to the community, Government or industry that would arise from this food regulatory measure.

- There are no other measures that would be more cost-effective than a variation to Standard 1.5.2 that could achieve the same end.

- There are no relevant New Zealand standards.

- There are no other relevant matters.

Decision

Approve the variation to Standard 1.5.2 – Food produced using Gene Technology, to include food derived from insect-protected corn line MIR162 in the Table to clause 2.

Reasons for Decision

A variation to Standard 1.5.2 to permit the sale and use of food derived from insect-protected corn line MIR162 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 MIR162;

- food derived from insect-protected corn line MIR162 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 foods derived from insect-protected corn line MIR162 will be required if novel DNA and/or protein is present in the final food;

- 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, a variation to Standard 1.5.2;

- there are no relevant New Zealand standards; and

- there are no other measures that would be more cost-effective than a variation to Standard 1.5.2 that could achieve the same end.

Consultation

Public submissions were invited on the Assessment Report between 11 April and 23 May 2008. Comments were specifically requested on the scientific aspects of this Application, in particular, information relevant to the safety assessment of food from insect-protected corn MIR162. A total of 24 submissions was received. A summary of these is provided in **Attachment 3** to this Report.

As this Application is being assessed as a general procedure, there was one round of public comment. Responses to the Assessment Report were used to develop this Approval Report for the Application. The main issues raised in public comments are discussed in the Approval 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. here 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. heir 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[9]) were fed GM soy containing the *epsps* gene. The amount of recombinant DNA that survived passage through the small bowel varied between the seven ileostomists, with a maximum of 3.7% recovered from the stoma of one individual. This rate of recovery was similar to an endogenous soy gene, suggesting the recombinant DNA was digested similarly to other plant DNA. The *epsps* gene could not be detected in faeces from subjects with intact digestive tracts, suggesting that any DNA surviving digestion in the upper GI tract is readily degraded in the large intestine. The study also found evidence of pre-existing transfer of a fragment of the *epsps* gene between GM soy and a small number of micro-organisms in the small intestine of the ileostomists. The authors speculated this had occurred prior to commencement of the study. There was no evidence of the intact *epsps* gene being transferred. In subjects with intact digestive tracts, none of the endogenous bacteria in the faeces were found to contain any *epsps* gene fragments from GM soy.

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

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

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

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

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

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;

- 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[10], is interesting in that the authors report that the GM soy-fed mice exhibited some slight but statistically significant ultrastructural differences in hepatocyte nuclei[11] relative to controls. Cells bearing slightly more irregularly shaped nuclei were postulated to be indicative of an increased metabolic rate and the slight increase in the number of nuclear pores was apparently suggestive of increased molecular trafficking between the nucleus and cytoplasm.

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

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

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

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

9. Conclusion

The transferred DNA in MIR162 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 MIR162; consideration of such issues is already implicit in the molecular characterisation component of the safety assessment.

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