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

22 May 2008
[8-08]

FIRST REVIEW REPORT

APPLICATION A592

FOOD DERIVED FROM GLYPHOSATE-TOLERANT SOYBEAN LINE MON 89788

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

CONTENTS

1. Introduction

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

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

2. Objectives of Review

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

3. Grounds for the Review requested by the Ministerial Council

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

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

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

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

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

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

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

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

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

4. Background

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

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

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

5. Conclusions from the Final Assessment Report

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

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

6. Issues addressed in First Review

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

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

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

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

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

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

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

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

6.4 Nucleic acids are broken down during food processing

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

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

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

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

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

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

In the Netherwood et al study, nineteen human volunteers (twelve with intact digestive tracts, seven with ileostomies[1]) were fed GM soy containing the *epsps* gene. The amount of recombinant DNA that survived passage through the small bowel varied between the seven ileostomists, with a maximum of 3.7% recovered from the stoma of one individual. This rate of recovery was similar to an endogenous soy gene, suggesting the recombinant DNA was digested similarly to other plant DNA. The *epsps* gene could not be detected in faeces from subjects with intact digestive tracts, suggesting that any DNA surviving digestion in the upper GI tract is readily degraded in the large intestine. The study also found evidence of pre-existing transfer of a fragment of the *epsps* gene between GM soy and a small number of micro-organisms in the small intestine of the ileostomists. The authors speculated this had occurred prior to commencement of the study. There was no evidence of the intact *epsps* gene being transferred. In subjects with intact digestive tracts, none of the endogenous bacteria in the faeces were found to contain any *epsps* gene fragments from GM soy.

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

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

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

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).

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

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

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

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

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

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

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

- DNA transferred through transduction or transformation may be susceptible to restriction by bacterial restriction endonucleases, which cleave double-stranded DNA;
- in the case of linear DNA, homology with sequences in the bacterial genome is necessary for integration to occur;
- to be expressed, the transferred DNA must contain an intact coding region and be associated with the appropriate bacterial expression signals:

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

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

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

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

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

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

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

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

The study by Malatesta et al (2002) on the ultrastructure of hepatocytes from mice fed GM soybean[2], is interesting in that the authors report that the GM soy-fed mice exhibited some slight but statistically significant ultrastructural differences in hepatocyte nuclei[3] relative to controls.

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

Cells bearing slightly more irregularly shaped nuclei were postulated to be indicative of an increased metabolic rate and the slight increase in the number of nuclear pores was apparently suggestive of increased molecular trafficking between the nucleus and cytoplasm.

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

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

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

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

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

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

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

6.9 Conclusion

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

7. Review Options

There are three options proposed for consideration under this Review:

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

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

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

8. Decision

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

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

The recommended option is Option 1.

Decision

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

9. Implementation and review

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

10. References

Aulrich, K., Reuter, T. & Flachowsky, G. (2002). The fate of foreign DNA in farm animals fed with genetically modified plants. *Proc. Soc. Nutr. Physiol*. **11:** 187 – 188.

Beever, D.E. & Kemp, C.F. (2000). Safety issues associated with the DNA in animal feed derived from genetically modified crops. A review of scientific and regulatory procedures. *Nutrition Abstracts & Reviews* **70:** 197 – 204.

Broll, H., Zagon, J., Butchske, A., Leffke, A., Spielgelberg, A., Böhme, H. & Flachowsky, G. (2005). The fate of transgenic inulin synthesizing potatoes in pigs. *J. Anim. Feed Sci*. **14 (Suppl. 1):** 333 – 336.

Carver, J.D. & Walker, W.A. (1995). The role of nucleotides in human nutrition. *Nutr. Biochem*. **6:** 58 – 72.

Codex (2004). Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants (CAC/GL 45-2003), Codex Alimentarius Commission, Rome.

Collas, P. & Aelstrom, P. (1997). Rapid targeting of plasmid DNA to zebrafish embryo nuclei by the nuclear localization signal of SV40 antigen. *Mol. Mar. Biol. Biotechnol*. **6:** 48 – 58.

Doerfler, W. (1991). Patterns of DNA methylation – evolutionary vestiges of foreign DNA inactivation as a host defence mechanism. A proposal. *Biol. Chem. Hoppe-Seyler* **372:** 557 – 564.

Doerfler, W., Orend, G., Schubbert, R., Fechteler, K., Heller, H., Wilgenbus, P. & Schroer, J. (1995). On the insertion of foreign DNA into mammalian genomes: mechanism and consequences. *Gene* **157:** 241 – 245.

Doerfler, W. & Schubbert, R. (1997). Fremde DNA im Saugersystem. *Deut. Arzt*. **94:** 51 – 52.

Ebbehoj, K.F. & Thomsen, P.D. (1991). Species differentiation of heated meat products by DNA hybridisation. *Meat Sci*. **30:** 221 – 234.

EFSA (2007). EFSA statement on the fate of recombinant DNA or proteins in meat, milk and eggs from animals fed with GM feed. European Food Safety Authority http://www.efsa.europa.eu/EFSA/Non_Scientific_Document/Annex_EFSA%20statement%20DNA%20proteins%20gastroint.pdf (accessed on 25 July, 2007).

Einspanier, R., Klotz, A., Kraft, J., Aulrick, K., Poser, R., Schwägele, F., Jahreis, G. & Flachowsky, G. (2001). The fate of forage plant DNA in farm animals: a collaborative case study investigating cattle and chicken fed recombinant plant material. *Eur. Food Res. Technol*. **212:** 129 134.

Flachowsky, G., Halle, I. & Aulrich, K. (2005). Long term feeding of Bt-corn – a 10 generation study with quails. *Arch. Anim. Nutr*. **59:** 449 – 451.

Flachowsky, G., Aulrich, K., Böhme, H. & Halle, I. (2007). Studies on feeds from genetically modified plants (GMP) – Contributions to nutritional and safety assessment. *Animal Feed Sci. Technol*. 133: 2 – 30.

FSANZ (2007). *Safety Assessment of Genetically Modified Foods*, Foods Standard Australia New Zealand, Canberra. http://www.foodstandards.gov.au/_srcfiles/GM%20FINAL%20Sept%2007L%20_2_.pdf

Gaye, P.B & Gillespie, S.H (2005). Antibiotic resistance markers in genetically modified plants : a risk to human health? *Lancet Infect. Dis*. **5:** 637 – 646.

Gorlick, D. & Mattaj, I.W. (1996). Nucleocytoplasmic transport. *Science* **271:** 1513 – 1518.

Guralnick, B., Thomsen, G. & Citovsky, V. (1996). Transport of DNA into the nuclei of *Xenopus* oocytes by a modified VirE2 protein of Agrobacterium. *Plant Cell* **8:** 363 – 373.

Hupfer, C., Hotzel, H., Sachse, K., Moreano, F. & Engel, K.H. (1998). Detection of the genetic modification in heat-treated products of Bt-maize by polymerase chain reaction. *Z. Lebensm. Unters. Forsch. A* **206:** 203 – 207.

Jonas, D.A., Elmadfa, I., Engel, K.-H., Heller, K.J., Kozianowski, G., A. König, A., Müller, D., Narbonne, J.F., Wackernagel, W. & Kleiner, J. (2001). Safety considerations of DNA in food. *Ann. Nutr. Metab.* **45:** 235 – 254.

Kärenlampi, S. (1996). Health effects of marker genes in genetically engineered food plants. Nordic Council of Ministers, Copenhagen, Denmark, 66 pp.

Levy, S. B. & Miller, R.V. (1989). *Gene transfer in the environment*. McGraw-Hill Publishing Company, New York.

Malatesta, M., Caporaloni, C., Gavaudan, S., Rocchi, M.B.L., Serafini, S., Tiberi, C. & Gazzanelli, G. (2002). Ultrastructural morphometrical and immunocytochemical analyses of hepatocyte nuclei from mice fed on genetically modified soybean. *Cell Struct. Funct*. **27:** 173 – 180.

Mazza, R., Soave, M., Morlacchini, M., Piva, G. & Marocco, A. (2005). Assessing the transfer of genetically modified DNA from feed to animal tissues. *Transgenic Res*. **14:** 775 – 784.

Mercer, D.K., Scott, K.P., Bruce-Johnson, W.A., Glover, L.A. & Flint, H.J. (1999). Fate of free DNA and transformation of the oral bacterium *Streptococcus gordonii* DL1 by plasmid DNA in human saliva. *Appl. Env. Microbiol*. **65:** 6 – 10.

Netherwood, T., Martín-Orúe, S.M., O'Donnell, A.G., Gockling, S., Graham, J., Mathers, J.C. & Gilbert, H.J. (2004). Assessing the survival of transgenic plant DNA in the human gastrointestinal tract. *Nature Biotechnol*. **22:** 204 – 209.

Nielsen, K.M., Bones, A.M., Smalla, K. & van Elsas, J.D. (1998). Horizontal gene transfer from transgenic plants to terrestrial bacteria – a rare event? *FEMS Microbiol. Rev*. **22:** 79 – 103.

Orend, G., Knoblauch, M., Kammer, C., Tjia, S.T., Schmitz, B., Linkwitz, A.,, Meyer G., Maas, J. & Doerfler, W. (1995). The initiation of de novo methylation of foreign DNA integrated into a mammalian genome is not exclusively targeted by nucleotide sequence. *J. Virol*. **69:** 1226 –1242.

Palacios, I., Hetzer, M., Adams, S.A. & Mattaj, I.W. (1997). Nuclear import of U snRNPs requires importin beta. *EMBO J*. **16:** 6783 – 6792.

Palka-Santini, M., Schwarz-Herzke, B, Hösel, M., Renz, D., Auerochs, S., Brondke, H. & Doerfler, W. (2003). The gastrointestinal tract as the portal of entry of foreign macromolecules: fate of DNA and proteins. *Mol. Gen. Genomics* **270:** 201 – 215.

Paul, J.H. (1992). Intergeneric natural plasmid transformation between Escherichia coli and a marine Vibrio species. In: *Genetic Transfers and Environment*, pp. 61 – 67 (Ed. M.J. Gauthier) Springer Verlag Berlin, Heidelberg, New York.

Popov, S., Rexach, M., Zybarth, G., Reiling, N., Lee, M.A., Ratner, L., Lane, C.M., Moore, M.S., Blobel, G. & Bukrinksy, M. (1998). Viral protein R regulates nuclear import of the HIV-1 pre-integration complex. *EMBO J.* **17:** 909 – 917.

Reuter, T. & Aulrich, K. (2003). Investigations on genetically modified maize (Bt-maize) in pig nutrition: fate of feed ingested foreign DNA in pig bodies. *Eur. Food Res. Technol.* **216:** 185 – 192.

Rosenberg, E., Koren, O., Reshef, L., Efrony, R., Zilber-Rosenberg, I. (2007). The role of microorganisms in coral health, disease and evolution. *Nature Reviews Microbiol.* **5:** 355 – 362.

Saphire, A.C., Guan, T., Schirmer, E.C., Nemerow, G.R. & Gerace, I. (2000). Nuclear import of adenovirus DNA in vitro involves the nuclear protein import pathway and hsc70. *J. Biol. Chem.* **275:** 4298 – 4304.

Schubbert, R., Lettmann, C. & Doerfler, W. (1994). Ingested foreign phage M13 DNA survives transiently in the gastrointestinal tract and enters the bloodstream of mice. *Mol. Gen. Genet.* **241:** 495 – 504.

Schubbert, R., Renz, D., Schmitz, B. & Doerfler, W. (1997). Foreign M13 DNA ingested by mice reaches peripheral leukocytes, spleen, and liver via the intestinal wall mucosa and can be covalently linked to mouse DNA. *Proc. Natl. Acad. Sci. USA* **94:** 961 – 966.

Schubbert, R., Hohlweg, U., Renz, D. & Doerfler, W. (1998). On the fate of orally ingested foreign DNA in mice: chromosomal association and placental transmission to the fetus. *Mol. Gen. Genet.* **259:** 569 – 576.

Straub, J.A., Hertel, C. & Hammes, W.P. (1999). The fate of recombinant DNA in thermally treated fermented sausages. *Eur. Food Res. Technol.* **210:** 62 – 67.

Tony, M.A., Butschke, A., Broll, A., Zagon, J., Halle, I., Dänicke, S., Schauzu, M., Hafes, H.M. & Flachowsky, G. (2003). Safety assessment of Bt-176 maize on broiler nutrition: degradation of maize DNA and its metabolic fate. *Arch. Anim. Nutr.* **57:** 235 – 252.

WHO (1991). Strategies for assessing the safety of foods produced by biotechnology, Report of a Joint FAO/WHO Consultation. World Health Organization, Geneva.

WHO (1993). Health aspects of marker genes in genetically modified plants, Report of a WHO Workshop. World Health Organization, Geneva.

Woodhams, D.C., Rollins-Smith, L.A., Alford, R.A., Simon, M.A. & Harris, R.N. (2007). Innate immune defenses of amphibian skin: antimicrobial peptides and more. *Animal Conservation* **10:** 425 – 428.

Zeimienowicz, A., Gorlich, D., Lanka, E., Hohn, B. & Rossi, L. (1999). Import of DNA into mammalian nuclei by proteins originating form a plant pathogenic bacterium. *Proc. Natl. Acad. Sci. USA* **96:** 3729 – 3733.

Attachments

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

Attachment 1

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

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

To commence: on gazettal

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

Food derived from glyphosate-tolerant soybean line MON 89788	

Attachment 2

Executive Summary and Reasons for Decision from the Final Assessment Report

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

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

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

Safety assessment

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

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

Labelling

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

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

Impact of regulatory options

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

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

Purpose

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

Decision

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

Reasons for Decision

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

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

Consultation

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