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

8-06
13 December 2006

FINAL ASSESSMENT REPORT

APPLICATION A549

FOOD DERIVED FROM HIGH LYSINE CORN LY038

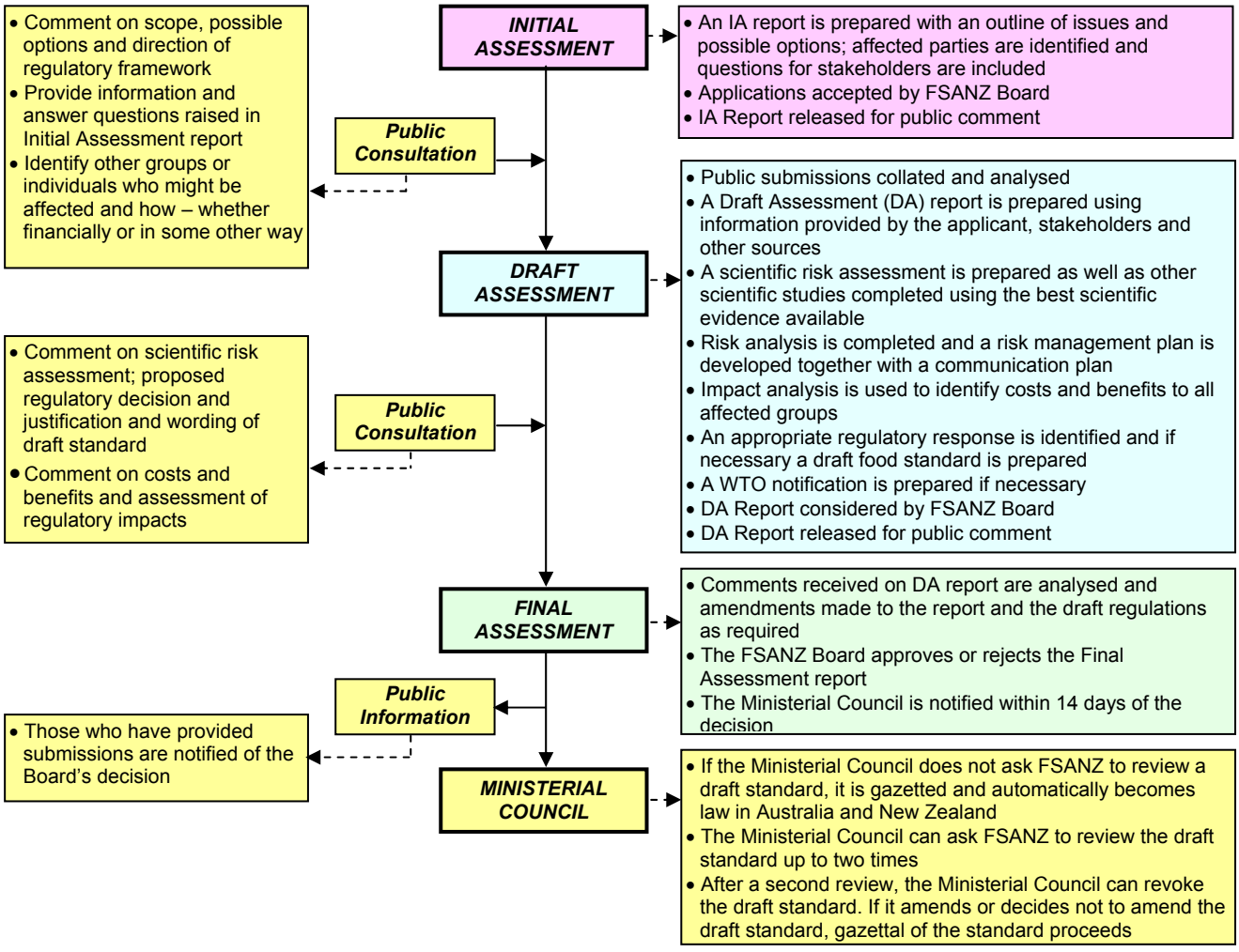
FOOD STANDARDS AUSTRALIA NEW ZEALAND (FSANZ)

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

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

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

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



Final Assessment Stage

FSANZ has now completed two stages of the assessment process and held two rounds of public consultation as part of its assessment of this Application. This Final Assessment Report and its recommendations have been approved by the FSANZ Board and notified to the Ministerial Council.

If the Ministerial Council does not request FSANZ to review the draft amendments to the Code, an amendment to the Code is published in the *Commonwealth Gazette* and the *New Zealand Gazette* and adopted by reference and without amendment under Australian State and Territory food law.

In New Zealand, the New Zealand Minister of Health gazettes the food standard under the New Zealand Food Act. Following gazettal, the standard takes effect 28 days later.

Further Information

Requests for further information on this Application and the assessment process should be addressed to the FSANZ Standards Management Officer at one of the following addresses:

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Assessment reports are available for viewing and downloading from the FSANZ website www.foodstandards.gov.au or alternatively paper copies of reports can be requested from FSANZ's Information Officer at info@foodstandards.gov.au including other general inquiries and requests for information.

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Executive Summary and Statement of Reasons

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 a genetically modified (GM) high lysine corn, corn line LY038. 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.

Corn line LY038 has been genetically modified to have higher than usual levels of the amino acid lysine. It contains the *cordapA* gene from *Corynebacterium glutamicum*, which results in the accumulation of lysine in the corn grain. Corn line LY038 is intended specifically for animal feed, however it is possible it may also enter the human food supply. For this reason FSANZ has conducted a safety assessment on food derived from high lysine corn LY038.

Food from corn line LY038 may enter Australia and New Zealand as imported products.

Safety assessment

FSANZ has completed a comprehensive pre-market safety assessment of food derived from corn line LY038 as required under Standard 1.5.2 of the Code. The assessment included consideration of: (i) the genetic modification to the plant; (ii) the potential toxicity and allergenicity of any new proteins; and (iii) the composition and nutritional adequacy of the food, including whether there had been any unintended changes. The potential impact of increased lysine was also assessed.

Although corn line LY038 is primarily intended for use as animal feed, the safety assessment conducted by FSANZ assumed the GM corn was intended for human food use and therefore was no different to the rigorous scientific assessment for any GM food.

No potential public health and safety concerns were identified and on the basis of all the available evidence, including detailed studies provided by the Applicant, it has been concluded that food derived from corn line LY038 is as safe and wholesome as food derived from other corn varieties.

Labelling

Food derived from corn line LY038 will be required to be labelled as genetically modified where novel DNA and/or protein are present in the final food. In addition to this, foods containing LY038 that have not been refined to remove the protein component (and hence lysine) will be required to be labelled with a statement informing consumers of the altered nutritional profile, that is, it contains increased lysine compared to other corn varieties.

Labelling addresses the requirement of section 10(1)(b) of the FSANZ Act; provision of adequate information relating to food to enable consumers to make informed choices.

Impact of regulatory options

Two regulatory options were considered in the assessment: either (1) no approval; or (2) approval of food derived from corn line LY038 based on the conclusions of the safety assessment.

Following a cost and benefit analysis of the potential impact of each of the options on the affected parties (consumers, the food industry and government), Option 2 is the preferred option as food from LY038 has been found to be as safe as food from other varieties of corn.

Consultation

A total of 214 submissions were received in response to the invitation to comment on the Initial and Draft Assessment Reports (200 on the Initial Assessment and 14 on the Draft Assessment). Issues raised in these submissions were considered in the Final Assessment.

A particularly significant submission was received in response to the Draft Assessment Report from the Centre for Integrated Research in Biosafety (INBI). This submission asserted that the assessment of high lysine corn has been inadequate, and raises a number of scientific points that in INBI's view should be considered in the assessment. FSANZ carefully considered each point raised and reiterates the conclusion of the safety assessment report that food derived from LY038 corn is as safe as foods from other varieties of corn.

External review was sought on the safety assessment report following the Draft Assessment. As this Application involves a novel gene and protein that FSANZ has not assessed before, it is standard practice for FSANZ to seek the opinion of external scientific experts. In general, the reviewers agreed with the conclusions of the safety assessment of LY038. Specific comments have been addressed in the safety assessment report or in this report.

FSANZ Decision

FSANZ agrees to amend Standard 1.5.2 of the Code to approve the sale and use of food derived from corn line LY038 in Australia and New Zealand.

Statement of Reasons

An amendment to Standard 1.5.2 of the Code to give approval to the sale and use of food derived from corn line LY038 in Australia and New Zealand is agreed on the basis of the available scientific evidence for the following reasons:

- the safety assessment did not identify any public health and safety concerns associated with the genetic modification used to produce corn line LY038;
- in terms of its safety for human consumption and nutritional adequacy, food derived from corn line LY038 is equivalent to food from other commercially available corn varieties. The only difference is the increase in lysine;
- labelling of certain food fractions derived from corn line LY038 will be required if novel DNA, novel protein and/or increased levels of lysine, are 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 amendment to the Code is of net benefit to both food producers and consumers; and

- the proposed draft variation to Standard 1.5.2 of the Code is consistent with the section 10 objectives of the FSANZ Act and the regulatory impact assessment.

The variation will come into effect on the date of gazettal.

1. Introduction

An Application was received from Monsanto Australia Limited on 25 October 2004 seeking approval for food derived from high lysine corn line LY038 under Standard 1.5.2 – Food produced using Gene Technology, in the Code.

The genetic modification involved the transfer of the following genes into the corn plant:

- the *cordapA* gene derived from *Corynebacterium glutamicum* which encodes a dihydrodipicolinate synthase (DHDPS). DHDPS is an enzyme that is involved in the lysine biosynthesis pathway. In plants, this enzyme is the rate-limiting step in lysine production as it is highly susceptible to lysine feedback inhibition. The bacterial DHDPS enzyme is >50 fold less sensitive than the plant enzyme, allowing the synthesis of lysine to continue even in the presence of high lysine levels; and

- the *nptII* gene (an antibiotic resistance gene), which was subsequently removed from the corn cells by recombination.

2. Regulatory Problem

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

Monsanto Australia Limited has developed a high lysine corn, known as line LY038, primarily for animal feed. Identity preservation methods will be used to segregate this product from conventional grain, however it is possible that a small percentage of LY038 grain will inadvertently be co-mingled with conventional corn and enter the human food supply. Monsanto Australia Limited has therefore applied to have Standard 1.5.2 amended to include food derived from corn line LY038 in the Table to clause 2.

3. Objective

The objective of the assessment is to determine whether it would be appropriate to amend the Code to approve the use of food derived from corn line LY038 under Standard 1.5.2. In developing or varying a food standard, FSANZ is required by its legislation to meet three primary objectives, which are set out in section 10 of the FSANZ Act. These are:

- the protection of public health and safety;

- the provision of adequate information relating to food to enable consumers to make informed choices; and

- the prevention of misleading or deceptive conduct.

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

- the need for standards to be based on risk analysis using the best available scientific evidence;

- the promotion of consistency between domestic and international food standards;
- the desirability of an efficient and internationally competitive food industry;
- the promotion of fair trading in food; and
- any written policy guidelines formulated by the Ministerial Council.

4. Background

The Applicant has developed GM corn plants that have higher than usual levels of lysine in the grain. These corn plants are referred to as corn line LY038 or 'MAVERA HVC with Lysine', where HVC stands for High Value Corn. The purpose of the modification was to produce corn grain with high levels of lysine to be used as animal feed. Conventional corn-soy based diets for swine and chicken are characteristically deficient in lysine and require the addition of supplemental lysine for optimal animal growth and performance.

Corn line LY038 contains one novel gene, *cordapA*, from *Corynebacterium glutamicum*, which encodes the enzyme dihydrodipicolinate synthase (DHDPS). This enzyme is involved in lysine biosynthesis. The bacterial DHDPS enzyme, unlike the plant DHDPS enzyme, is not sensitive to lysine feedback inhibition, so lysine biosynthesis will continue in the presence of high levels of free lysine.

The levels of free lysine in corn line LY038 are expected to be in the range of 1000 to 2500 parts per million (ppm) in the grain, compared to <100 ppm in conventional corn grain. The total lysine level in conventional corn, most of which is present as protein-incorporated lysine, typically ranges from 2500 to 2800 ppm on a dry weight basis. Therefore in LY038 the expected total lysine would range from 3500 ppm to 5300 ppm. The quantity of protein-incorporated lysine in corn line LY038 is expected to be the same as in conventional corn.

High lysine corn line LY038 is intended for use as field corn for animal feed and will not be bred into other types of corn such as sweet corn and popcorn. As LY038 will not be grown in Australia and New Zealand, the only source of LY038 corn in our food supply would be in imported products. The types of food products that could contain ingredients derived from corn line LY038 in the case of inadvertent co-mingling are: margarine, cooking oil and baking and frying fats; various sweeteners including high fructose, dextrose, and maltodextrins; corn grain used as an additive; flaking grits used almost exclusively in the manufacture of corn flakes; fine grits utilised by the snack, breakfast cereal and brewing industries; coarse grits eaten as a breakfast food; corn flour; dried-milled corn products used as a substrate for brewing beer; and corn grits and whole kernels used to produce many distilled hard liquors.

Domestic production of corn in Australia and New Zealand is supplemented by the import of a small amount of corn-based products, largely as high-fructose corn syrup, which is not currently manufactured in either Australia or New Zealand.

Corn syrup is a processed product, is not expected to contain amino acids and would therefore not have increased levels of lysine.

LY038 corn has been approved for food and feed use in Canada. Applications for approval have been made in the United States, Japan, the European Union and Argentina and are still under consideration by the relevant regulatory bodies.

5. Relevant Issues

5.1 Safety assessment of food from corn line LY038

Food from corn line LY038 has been evaluated according to the safety assessment guidelines prepared by FSANZ[1]. The safety assessment included the following:

- a characterisation of the genetic modification to the plant;
- characterisation of any novel proteins, including their potential toxicity and allergenicity; and
- a comparative analysis of the key constituents of corn line LY038.

The Applicant submitted a comprehensive data package in support of their application, including studies on the molecular characterisation of the insert in line LY038, the toxicity and potential allergenicity of cDHDPS, and compositional analyses of food derived from corn line LY038. The nutritional impact of the increased lysine content was also assessed. In addition to information supplied by the Applicant, FSANZ also had regard to other available information, including from the scientific literature, general technical information, independent scientists, other regulatory agencies and international bodies, and the general community.

No potential public health and safety concerns were identified in the assessment of food derived from corn line LY038. Therefore, on the basis of all the available evidence, including detailed studies provided by the Applicant, it has been concluded that food derived from corn line LY038 is as safe and wholesome as food derived from other corn varieties. The full safety assessment report is at **Attachment 2** to this document.

5.2 Nutritional impact of corn line LY038

The potential nutritional impact of LY038 corn on the human food supply has been assessed. The two nutrition issues of primary importance for public health and safety are:

- whether the differences in the nutritional profile of LY038 corn compared to conventional corn are significant enough to have an impact on Australian and New Zealand diets; and
- whether LY038 corn will contain increased levels of substances (other than lysine) that may interfere with the intake or bioavailability of other nutrients.

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

5.2.1 The Amino Acid Profile of LY038 Corn Compared to Conventional Corn

The intended change produced in LY038 corn by the genetic modification was the increase in levels of the essential amino acid lysine. However, traditionally, corn does not contain a significant amount of lysine compared to the other dietary sources such as animal products, and although LY038 has increased lysine, the amount of lysine present would not have a significant effect on the human diet.

The human body uses amino acids from digested protein most effectively when the correct proportions of essential amino acids are provided in the diet, however this is more important when the intake of protein is only just sufficient to meet requirements. If there is a reduction in the intake of one amino acid compared to others, then protein synthesis by the body is reduced accordingly, and the intakes of other essential amino acids will be wasted via expenditure through energy metabolism[2]. However, in the case of adequate protein intakes, this is not an issue.

In the diverse diets of Australian and New Zealand populations, foods can be combined to ensure that amino acids are optimally supplied to meet protein synthesis needs[3]. However this combination assumes that the foods have their standard amino acid profile. Should the LY038 variation result in a significantly different amino acid profile (aside from lysine) compared to conventional corn, then this genetic modification may pose a risk to population nutrition.

The Applicant has provided information comparing LY038 corn to a closely related control corn line, LY038(-), both grown in the same locations. This information is presented in Attachment 2 – Safety Assessment Report.

Four amino acids are significantly reduced in LY038 corn compared to its control, and with the exception of glutamic acid, all are essential amino acids (histidine, isoleucine and phenylalanine). However, when compared to conventional corn data, the reductions of these amino acids in LY038 corn are shown to remain within the normal variation observed in corn grain.

Due to the change in lysine levels, there is also the possibility of the occurrence of different Maillard reactions, which can make lysine unavailable by forming complexes. This is not considered to be of concern as the generally high protein intake by the Australian and New Zealand populations means that the additional lysine in LY038 is unnecessary for health. Furthermore, much of the intake of corn is in the form of processed products (e.g. corn syrup) that contain negligible amounts of protein.

5.2.2 The Fat, Carbohydrate, Vitamin and Mineral Contents of LY038 Corn Compared to Conventional Corn

The only intended change in the nutritional profile of LY038 corn grain is the increased lysine content. Therefore, the greatest nutritional risks are likely to manifest themselves through changes to protein / amino acid contents.

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[2] Wardlaw, G.M. and Insel, P.M. (1996) Perspectives in Nutrition. 3rd ed, Mosby-Year Book Inc., Sydney.
[3] FAO/WHO (1991) *Protein Quality Evaluation*. Food and Nutrition Paper Series 51, Food and Agriculture Organization of the United Nations, Rome.

However for completeness, FSANZ has also assessed whether there are any potential nutritional issues from changes in the fatty acid, total carbohydrate, vitamin or mineral content of LY038 corn.

The Applicant's data comparing LY038 corn to a control corn crop shows that of the non-protein nutrients in corn (excluding dietary fibre – see section 5.2.3 below), linoleic acid, vitamin E, and calcium are significantly reduced. However, when compared to their respective international content values for conventional field corn, these nutrients are well within the normal variation.

5.2.3 Nutritional Inhibitors within LY038

There is the risk that with changes in the lysine content of LY038 corn, there will be additional or increased intakes of substances that may have an inhibitory effect on the digestion and uptake of nutrients by the human body.

Corn contains phytic acid, that can bind minerals and reduce their uptake by the body. The Applicant has provided data on the phytic acid content of LY038 compared to LY038(-), showing that there is a non-significant decrease in the phytic acid content of LY038 (0.68±0.04 and 0.77±0.04 % dry wt respectively). The phytic acid content of LY038 is also within the international range for field corn of 0.45 to 1.0 % dry wt (OECD, 2002).

The Applicant's data also show that the plant lysine metabolic by-products, α-aminoadipic acid and saccharopine, are increased within LY038 compared to LY038(-). However, FSANZ has not been able to identify any adverse nutritional impacts from increased intakes of these substances in the available scientific literature.

5.2.4 Conclusion

The compositional data on LY038 corn supplied by the Applicant indicates the following:

- LY038 corn has a similar nutritional profile to conventional corn, with the exception of increased free lysine content. It should be noted that the main product produced from corn that is imported into Australia and New Zealand is high fructose corn syrup, which would contain very little, if any, protein; and

- the genetic modifications of LY038 corn do not significantly increase the level of substances that have the potential to interfere with the intake or bioavailability of nutrients.

Therefore, any potential consumption of LY038 corn by humans will not adversely affect the overall quality of protein/amino acid, vitamin, mineral, fat or fibre intakes of Australian and New Zealand populations.

5.3 Labelling

Under Standard 1.5.2, GM food or ingredients must be labelled if novel DNA and/or protein are present in the final food or where the food has altered characteristics. Therefore, food derived from corn line LY038 will be required to be labelled as genetically modified where novel DNA and/or protein are present in the final food.

In addition to this, foods containing LY038 that have not been refined to remove the protein component (and hence lysine) will be required to be labelled with a statement informing consumers of the altered nutritional profile, that is, it contains increased lysine compared to other corn varieties.

5.4 Issues arising from public submissions

In addition to the specific issues addressed below, FSANZ has also developed a Fact Sheet: *Frequently Asked Questions on Genetically Modified Foods – August 2002*, which responds to many of the general issues raised in connection with GM foods. The Fact Sheet may be obtained from the FSANZ website[4].

5.4.1 Concern that the use of LY038 corn may not be restricted to animal feed once food approval is given.

Friends of the Earth (New Zealand) expressed concern that if LY038 corn is approved as food in Standard 1.5.2, its use may not be restricted to animal feed and growers may intentionally use it in food products.

5.4.1.1 FSANZ response

High lysine corn LY038 is intended for use as field corn for animal feed. The Applicant states that identity preservation methods will be used to segregate the product from other commercial corn grain and it will not be used in conventional breeding programs for other types of maize such as sweet corn, which is used predominantly as human food. Given the nature of the genetic modification, FSANZ considers there would be no sound commercial reasons to use high lysine corn in significant amounts in grain destined for human consumption.

However, FSANZ has assessed high lysine corn as if it were intended for human consumption. The prevailing view of government is that GM lines developed as animal feed should also undergo assessment as food for humans, recognising that co-mingling of commercial grains does occur. This approach has two beneficial outcomes: firstly, there is a requirement for a pre-market safety assessment for lines developed primarily as animal feed, and secondly, but more importantly, it ensures the protection of public health and safety should the product enter the human food supply either inadvertently or as a result of co-mingling practices.

5.4.2 Approval by other regulatory agencies

Friends of the Earth (New Zealand) expressed concern that LY038 has not been approved as food elsewhere in the world.

5.4.2.1 FSANZ Response

Since Draft Assessment of this Application, LY038 has been approved for use in food and feed in Canada by the agency responsible for food safety, Health Canada.

[4] www.foodstandards.gov.au/mediareleasespublications/factsheets/factsheets2002/index.cfm

5.4.3 Conduct of the broiler chicken feeding study

The South Australian Department of Health suggested that further analysis (e.g. histopathology) of the broilers in the feeding study could have lent weight to the safety assessment of high lysine corn.

5.4.3.1 FSANZ Response

The aim of feeding studies using broiler chickens, such as the one referred to above, is to determine the effects of a new GM food on the growth and wellbeing of animals that would traditionally be fed the conventional comparator (i.e. conventional corn). Detailed analyses such as histopathology, while routine in traditional toxicological studies, are not generally conducted in this kind of feeding study, and would not be expected to provide meaningful results in the absence of historical data on which to base a comparison.

5.4.4 Concern that the novel DNA may enter the tissues of animals that consume LY038 corn

One submitter expressed concern that novel DNA in GM feeds may enter the tissues of animals feeding on the GM product. Concern was also expressed that these products would not be required to be labelled as genetically modified.

5.4.4.1 FSANZ Response

Fragments of plant DNA have been detected in animal tissues, including milk, but there is no basis to suppose that the new DNA poses a hazard. A recent report concluded *No intact or immunologically reactive fragments of transgenic plant proteins or deoxyribonucleic acid (DNA) have been detected in samples of meat, milk, eggs, lymphocytes, blood, and organ tissue from production animals fed biotechnology-derived crops modified for agronomic input traits.*[5] Humans and animals have been consuming plant DNA for generations without adverse effects.

Foods derived from animals fed GM feed are not required to be labelled as genetically modified as the animal itself does not become genetically modified or changed in any way by eating GM feed. Studies conducted to date indicate that there are no biologically relevant differences between animal products such as meat, milk and eggs, whether they are produced from animals fed GM or conventional feed.

5.4.5 Allergenicity studies with CSIRO GM peas

A number of submitters expressed concern about the potential for new GM foods to be allergenic, referring to a recently published study on work conducted by the CSIRO where a GM field pea was found to cause an allergic response in rodents.

[5] Council for Agricultural Science and Technology (CAST). 2006. *Safety of Meat, Milk, and Eggs from Animals Fed Crops Derived from Modern Biotechnology*. Issue Paper 34. CAST, Ames, Iowa. http://www.cast-science.org

5.4.5.1 FSANZ Response

The potential allergenicity of high lysine corn LY038 has been assessed using a step-wise approach as advocated by the Codex Alimentarius Commission. The results of this series of studies indicated that the novel protein, cDHDPS, is unlikely to be a potential allergen.

In regard to the CSIRO GM field pea trials, on the basis of the available information, FSANZ does not believe it is possible to draw any conclusions about the potential for the modified form of the novel protein in these peas, an alpha-amylase inhibitor, to be a human food allergen.

At present there is no single test that can be used to determine if a new protein is likely to be allergenic to humans. The internationally accepted approach is to use a variety of data and information, which when considered together can be used to reach a conclusion about potential allergenicity of a new protein. The various animal models that are available are not considered to be sufficiently well developed or validated to use at the present time for this assessment.

While FSANZ does not require that animal models be used for the assessment of potential allergenicity, it is important to note that the modified form of the alpha-amylase inhibitor protein would have been readily identified by the types of protein characterisation studies that are routinely undertaken with all novel proteins and submitted to FSANZ for assessment. Such a finding would have automatically triggered further testing of the protein.

5.4.6 Studies conducted to support the safety of LY038 corn

Two submitters questioned whether the FSANZ data requirements for studies submitted by the Applicant to support the safety of high lysine corn line LY038 were adequate.

5.4.6.1 FSANZ response

FSANZ has completed a scientific assessment of the safety of food derived from high lysine corn line LY038. The studies evaluated by FSANZ are detailed in the Safety Assessment (Attachment 2).

Approvals of GM foods for human consumption by FSANZ are based on comprehensive, rigorous and science-based pre-market safety assessments that are consistent with international guidelines developed by the Food and Agricultural Organization of the United Nations, World Health Organization, the Organisation for Economic Cooperation and Development and Codex Alimentarius Commission. These international guidelines have been adopted by food regulators worldwide, including in the US, Canada, Japan, Europe and many other countries.

5.4.7 Institute for Integrated Research in Biosafety (INBI) Submission

FSANZ received a detailed submission on the Draft Assessment Report for Application A549 from the Centre for Integrated Research in Biosafety (INBI, previously the New Zealand Institute of Gene Ecology, NZIGE).

The submission, which includes 94 recommendations in relation to the safety assessment of food from high lysine corn, follows comments previously submitted by the NZIGE on the Initial Assessment Report. These comments were addressed by FSANZ at Draft Assessment.

The current submission from INBI asserts the following:

1. The scientific studies on LY038 do not prove it to be as safe as conventional corn;
2. LY038 has a substantially different potential to create food hazards during cooking;
3. Hybrids with LY038 could create significant additional food hazards;
4. The novel protein has no history of safe use;
5. LY038 has been tested as an animal feed, not a human food;
6. FSANZ has accepted a standard of evidence of safety that is below what it could request under international guidelines; and
7. A recommendation to amend the Code does not follow from a case-by-case assessment.

After consideration of the evidence, INBI expresses the view that:

- too much legitimate scientific uncertainty exists;
- there is considerable evidence of probable harm in comparison to conventional corn;
- the recommendation is inconsistent with Codex;
- more studies should be requested from the Applicant;
- any approval for high lysine corn should be restricted to food derived directly from the specific line evaluated (LY038) and not include food from hybrid lines; and
- FSANZ should impose an actively managed post-market monitoring program.

5.4.3.1 FSANZ response

FSANZ undertook a comprehensive analysis of the INBI submission, and also evaluated the references cited in the submission for their potential to inform the safety assessment and impact on the overall conclusions.

During the analysis of the INBI submission, FSANZ observed it contained a number of inconsistencies and inaccuracies with regard to the discussion and reporting of the scientific literature. For example, while FSANZ has been criticised by INBI for deviating from the Codex guideline, INBI have repeatedly suggested the use of experimental techniques that are not endorsed by Codex or other intergovernmental organisations, and which have not been validated for the purpose of safety assessment (e.g. RNA microarray). While advocating the use of methods which still require development and are yet to be validated, INBI criticises well-established methodologies such as bioinformatics which are endorsed by Codex and the FAO/WHO as part of an overall strategy for assessing potential allergenicity.

FSANZ has also clarified a number of factual errors in the INBI submission, some journal articles cited by INBI as evidence supporting a particular view have been misinterpreted and the results and conclusions drawn by the author of the journal article are contrary to those represented in the INBI submission.

FSANZ has considered the scientific issues within the context of assessing food safety, and prepared a detailed response to each of the recommendations.

While FSANZ acknowledges that certain points raised in the INBI submission would be of academic interest, the requirements for additional data are considered unnecessary and, for the most part, impractical to demonstrate the safety of food.

On the basis of the current evidence, FSANZ reaffirms the conclusions of the safety assessment; food from high lysine corn LY038 is considered as safe for human consumption as food from conventional corn varieties.

The detailed responses to each recommendation made by INBI are included in Attachment 4 to this report.

5.5 External review of safety assessment

The safety assessment report (at **Attachment 2**) for this Application was submitted to two external reviewers for expert comment.

Both reviewers generally considered that the safety of food derived from corn line LY038 was adequately demonstrated by the information presented in the safety assessment report prepared by FSANZ. Specific comments raised by the reviewers were incorporated into the safety assessment report where appropriate.

6. Regulatory Options

6.1 Option 1 – do not approve food from high lysine corn line LY038

Maintain the *status quo* by not amending the Code to approve the sale and use of food derived from corn line LY038.

6.2 Option 2 – approve food from high lysine corn line LY038

Amend Standard 1.5.2 of the Code to permit the sale and use of food derived from corn line LY038, with or without listing special conditions in the Table to clause 2 of Standard 1.5.2.

7. Impact Analysis

7.1 Affected parties

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

7.2 Impact analysis

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

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

The following is an assessment by FSANZ of the potential costs and benefits of the two regulatory options. This is based on information supplied by the Applicant, issues raised in the two rounds of public comment on the Application and experience FSANZ has gained from consideration of previous applications relating to GM foods.

7.2.1 Option 1

Consumers: No impact on consumers wishing to avoid GM foods, as food from corn line LY038 is not currently permitted in the food supply.

Loss of consumer confidence in food supply if LY038 accidentally enters the food supply.

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

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

Potential cost to industry if LY038 corn becomes co-mingled with varieties of corn used in food, and corn products need to be recalled.

7.2.2 Option 2

Consumers: Benefit as consumers can maintain confidence in the food supply if LY038 becomes co-mingled with other corn varieties.

Other varieties of GM corn have already been approved so the cost to consumers wishing to avoid GM food (e.g. a potential restriction of choice of products, or increased prices for non-GM food) is likely to be low. Many food products containing LY038 grain will be required to be labelled.

Government: Benefit that if LY038 corn were to inadvertently enter the human food supply, this Application will ensure food imports containing LY038 corn comply with the Code. This would ensure that there is no potential for trade disruption on regulatory grounds.

This decision may impact on monitoring resources as some foods derived from corn line LY038 will be required to be labelled as genetically modified and as having increased lysine levels. However, this cost would not necessarily be avoided by adopting Option 1 as monitoring for the illegal presence of LY038 could be necessary.

Industry: Possible benefit if LY038 is commingled with other corn varieties as no regulatory action would need to be taken and so costs from this (e.g. product recall) are likely to be negligible.

Possible cost to food industry as some foods derived from corn line LY038 will be required to be labelled as genetically modified and as having increased lysine levels.

7.2.3 Discussion

As food from LY038 corn has been found to be as safe as food from other varieties of corn, option 1 is likely to be inconsistent with Australia and New Zealand's WTO obligations. This option would also offer very little benefit to those consumers wishing to avoid GM foods, as food from other GM corn varieties is already permitted in the food supply.

Option 2 is the preferred option as LY038 has been found to be safe for human consumption, and provides the benefit that approval of this line may prevent problems in the future if LY038 were to enter the food supply.

The proposed amendment to Standard 1.5.2 of the Code, giving approval to food from corn line LY038, is therefore considered appropriate.

8. Consultation

8.1 Public Consultation

The Initial Assessment of this Application was advertised for public comment between 15 December 2004 and 9 February 2005. A total of 200 submissions were received during this period. On completion of the Draft Assessment, further public comment was invited between 22 March 2006 and 3 May 2006. FSANZ received 14 submissions. All submissions have been summarised in **Attachment 3** to this Final Assessment Report.

FSANZ has now completed the assessment of the Application, including a safety evaluation of the food and consideration of comments received in two rounds of public consultation. FSANZ will notify the outcomes of the Final Assessment Report to the Ministerial Council.

8.2 World Trade Organization (WTO)

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

Guidelines for assessing the safety of GM foods have been developed by the Codex Alimentarius Commission and have the status of standards for WTO purposes. The proposed amendment to the Code to allow food derived from corn line LY038 may be of interest to other WTO member nations because it pertains to the safety of GM food and is likely to have a liberalising effect on international trade.

For these reasons, FSANZ notified the WTO under the Sanitary and Phytosanitary Measures (SPS) Agreement. No responses were received in response to the notification.

9. Decision

FSANZ agrees to amend Standard 1.5.2 of the Code to approve the sale and use of food derived from corn line LY038 in Australia and New Zealand.

Statement of Reasons

An amendment to Standard 1.5.2 of the Code to give approval to the sale and use of food derived from corn line LY038 in Australia and New Zealand is agreed on the basis of the available scientific evidence for the following reasons:

- the safety assessment did not identify any public health and safety concerns associated with the genetic modification used to produce corn line LY038;
- in terms of its safety for human consumption and nutritional adequacy, food derived from corn line LY038 is equivalent to food from other commercially available corn varieties. The only difference is the increase in lysine;
- labelling of certain food fractions derived from corn line LY038 will be required if novel DNA, novel protein and/or increased levels of lysine, are 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 amendment to the Code is of net benefit to both food producers and consumers; and
- the proposed draft variation to Standard 1.5.2 of the Code is consistent with the section 10 objectives of the FSANZ Act and the regulatory impact assessment.

10. Implementation and review

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

ATTACHMENTS

Attachments to the Assessment Report include:

1. Draft variation to the *Australia New Zealand Food Standards Code*
2. Safety assessment report
3. Submission summary
4. FSANZ response to submission from the Centre for Integrated Research in Biosafety

ATTACHMENT 1

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

To commence: on gazettal

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

Food derived from high lysine corn line LY038	Unless the protein content has been removed as part of a refining process, the label on or attached to a package of a food derived from high lysine corn line LY038 must include a statement to the effect that the food has been genetically modified to contain increased levels of lysine.

ATTACHMENT 2

SAFETY ASSESSMENT REPORT

APPLICATION A549 – FOOD DERIVED FROM HIGH LYSINE CORN LY038.

SUMMARY AND CONCLUSIONS

Background

Food derived from genetically modified (GM) corn line LY038 has been assessed for its safety for human consumption. This corn line has been genetically modified to contain higher levels of free lysine compared to conventional varieties of corn and is intended for use as an animal feed. However, as some LY038 corn may inadvertently enter the human food supply, FSANZ has completed a comprehensive safety assessment of food derived from this corn variety. If approved, food derived from LY038 corn may enter the Australian and New Zealand food supply as imported food products.

A number of criteria have been addressed in the safety assessment including: a characterisation of the transferred genes, their origin, function and stability; changes at the DNA, protein and whole food levels; compositional analyses; evaluation of intended and unintended changes; and the potential for the newly expressed proteins to be either allergenic or toxic to humans.

History of Use

Corn (*Zea mays L)*, otherwise known as maize, is the world's third leading cereal crop, behind wheat and rice, and is grown in over 25 countries worldwide. Corn-derived products are routinely used in a large number and diverse range of foods and have a long history of safe use. Although LY038 will be grown as a high value animal feed, a small percentage of this corn may enter the food supply.

Description of the Genetic Modification

The high lysine trait in LY038 corn is conferred by a single novel gene derived from the bacterium *Corynebacterium glutamicum*. The novel gene (*cordapA*) encodes a bacterial version of the enzyme dihydrodipicolinate synthase (DHDPS). The bacterial DHDPS enzyme (cDHDPS), unlike the plant DHDPS enzyme, is not sensitive to lysine feedback inhibition, so lysine biosynthesis will continue in the presence of high levels of free lysine. Detailed molecular and genetic analyses of this corn indicate that the transferred gene is stably integrated into the plant genome at one insertion site and is stably inherited from one generation to the next.

Characterisation of Novel Protein

Corn line LY038 expresses one novel protein, cDHDPS. This protein is expressed at varying levels in different parts of the corn plant, but is most highly expressed (26 µg/g dry weight) in the grain.

SDS-PAGE and Western blotting techniques indicated that the cDHDPS protein expressed in LY038 corn was of the expected size. N-terminal sequencing and MALDI-TOF mass spectrometry further confirmed that the desired protein was expressed in LY038. LY038 produced cDHDPS does not appear to be glycosylated.

An acute oral toxicity study has been conducted in mice with cDHDPS giving no evidence of toxicity at a dose of 800 mg/kg body weight. Potential allergenicity was assessed using an integrated, step wise approach which included consideration of the source of protein, amino acid sequence similarity to known allergens, and digestibility in simulated gastric conditions. When considered together, these data did not indicate any potential for allergenicity.

Comparative Analyses

Compositional analyses were done to establish the nutritional adequacy of grain from LY038 corn, and to compare it to a non-transgenic control line and commercial varieties of corn. The constituents measured were protein, fat, carbohydrate, ash, moisture, fibre, fatty acids, amino acids, vitamins, minerals, secondary metabolites (including metabolites involved in the synthesis and catabolism of lysine) and anti-nutrients.

No differences of biological significance were observed between LY038 grain and the control and commercial grain, other than those expected including increased lysine and related metabolites. The increased levels of lysine observed, along with lysine catabolites, do not raise any human health and safety concerns. Such levels are found in a number of other food plants therefore humans have been exposed to similar levels without any adverse effects.

Nutritional Impact

A 42-day feeding study in broiler chickens was conducted with LY038 and control grain (supplemented with lysine and non-supplemented). No adverse effects were observed in the chickens fed LY038 grain. Growth and feed efficiency was comparable between birds fed LY038 grain and lysine supplemented conventional corn grain. Carcass measurements were also equivalent between these birds. Growth and carcass measurements of the birds fed either lysine-supplemented diets or the LY038 corn diet were greater than birds fed non-supplemented conventional corn diets, as would be expected.

A 90-day feeding study in rats was also conducted. The Applicant states that no test related adverse effects were observed in rats fed LY038 corn at levels of up to 33% of the diet.

Conclusion

No potential public health and safety concerns have been identified in the assessment of food produced from LY038 corn. On the basis of the data provided in the present application, and other available information, food produced from LY038 corn has been significantly changed with respect to its lysine content, but can be considered as safe and as wholesome as food produced from other corn varieties.

1. INTRODUCTION

Monsanto Australia Limited has submitted an application to Food Standards Australia New Zealand (FSANZ) to vary Standard 1.5.2 – Food Produced Using Gene Technology – in the Code, to include food from a new genetically modified (GM) corn variety. The GM corn variety is designated LY038 and the intended product name for this corn is 'MAVERA HVC with Lysine', where HVC stands for High Value Corn.

LY038 corn produces grain with high levels of lysine that is intended to be used as animal feed. Conventional corn-soy based pig and chicken feeds are characteristically deficient in lysine and require the addition of supplemental lysine for optimal animal growth and performance.

Corn line LY038 contains one novel gene, *cordapA*, from *Corynebacterium glutamicum*, which encodes the enzyme dihydrodipicolinate synthase (DHDPS). This enzyme is involved in lysine biosynthesis. The bacterial DHDPS enzyme (cDHDPS), unlike the plant DHDPS enzyme, is not sensitive to lysine feedback inhibition, so lysine biosynthesis will continue in the presence of high levels of free lysine.

The *nptII* gene (an antibiotic resistance gene) was also transferred into LY038 corn, but then subsequently removed from the corn cells by recombination. No additional genes are present in LY038 corn.

The levels of free lysine in corn line LY038 are expected to be in the range of 1000 to 2500 parts per million (ppm) in the grain, compared to <100 ppm in conventional corn grain. The total lysine level in conventional corn, most of which is present as protein-incorporated lysine, typically ranges from 2500 to 2800 ppm on a dry weight basis. Therefore in LY038 corn the expected total lysine would range from 3500 ppm to 5300 ppm. The quantity of protein-incorporated lysine in corn line LY038 is expected to be the same as in conventional corn.

High lysine corn line LY038 is intended for use as animal feed and the Applicant does not intend to breed it into other types of corn such as sweet corn and popcorn. As LY038 will not be grown in Australia and New Zealand, the only source LY038 in our food supply would be in imported products. The types of food products that might contain corn line LY038 are: margarine, cooking oil and baking and frying fats; various sweeteners including high fructose, dextrose, and maltodextrins; corn grain used as an additive; flaking grits used almost exclusively in the manufacture of corn flakes; fine grits utilised by the snack, breakfast cereal and brewing industries; coarse grits eaten as a breakfast food; corn flour; dried-milled corn products used as a substrate for brewing beer; and corn grits and whole kernels used to produce many distilled hard liquors.

Many of these products are highly refined (e.g. corn oil and corn syrup) and would be unlikely to contain increased levels of lysine.

2. HISTORY OF USE

2.1 Donor Organisms

Corynebacterium glutamicum

Corynebacterium glutamicum (*Brevibacterium flavum*), is a non-pathogenic species of coryneform bacteria, which are rod-shaped, nonsporulating Gram-positive bacteria that are widely distributed in nature.

Strains of *C. glutamicum* have been used for commercial production of a number of amino acids including lysine. Commercial lysine production is primarily via fermentation of *C. glutamicum* strains that express dihydrodipicolinate synthase (cDHDPS) as this bacterial enzyme has decreased sensitivity to lysine feedback inhibition.

C. glutamicum is not known to cause disease in humans or animals.

2.2 Host Organism

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

The majority of grain and forage derived from maize is used as animal feed, however maize also has a long history of safe use as food for human consumption. The grain can be processed into industrial products such as ethyl alcohol (by fermentation), and highly refined starch (by wet-milling) to produce starch and sweetener products. In addition to milling, the maize germ can be processed to obtain corn oil and numerous other products (White and Pollak, 1995). However, because LY038 corn is not intended for food, human consumption is expected to be extremely low.

The germplasm that was the recipient of the transgenes in LY038 corn is a publicly available inbred line of maize, H99. This inbred line was used because it responds particularly well to particle bombardment and tissue culture regeneration.

3. DESCRIPTION OF THE GENETIC MODIFICATION

3.1 Method used in the genetic modification

Corn LY038 was produced by a particle acceleration methodology, using an *XhoI* linear fragment of DNA from plasmid vector PV-ZMPQ76. This fragment contained the *cordapA* gene as well as the *nptII* gene, which encodes the enzyme neomycin phosphotransferase II (NPTII) and was used as a selectable marker. NPTII confers resistance to aminoglycoside antibiotics including paromomycin. Plants regenerated from the callus tissue cells growing in the presence of paromomycin were assayed for the presence of the *cordapA* gene using standard PCR methodology and only positive plants were propagated.

Positive plants underwent a series of conventional crosses, which are shown in Figure 1. Although the original transformants contained the *nptII* gene, it was subsequently removed from the plant genome using the Cre-*lox* site-specific recombination system. The *nptII* gene had been designed so that it was flanked on either side by a copy of the 34-base pair (bp) *lox*P site. Regenerated plants that were positive for the *cordapA* gene were crossed with maize plants expressing Cre recombinase. These plants contain the *cre* gene, which had been introduced by *Agrobacterium* mediated gene transfer. In the resulting hybrid, Cre recombinase binds to the *lox*P sites on either side of the *nptII* gene, catalysing a crossover resulting in the excision of the *nptII* gene. This process results in a single *lox*P site remaining in the plant genome. The excised *nptII* gene (which is excised as circular extra-genomic DNA) does not contain an origin of replication and was subsequently lost, most likely through cell division.

Following crossing with the *Cre* line to remove the *nptII* gene, plants were selfed to give the F2 generation. F2 plants were screened by PCR to identify a single plant that was positive for *cordapA* and negative for *cre* and *nptII*. This plant was selfed to produce the F3 generation, a population segregating for the presence/absence of the *cordapA* gene. An F3 plant positive for *cordapA* was designated LY038 and a sibling plant negative for the same gene was designated as the negative segregant LY038(-).

Further conventional crossing then was conducted between each of LY038 and LY038(-) and other conventional corn varieties.

Figure 1: Diagrammatic representation of LY038 corn breeding tree

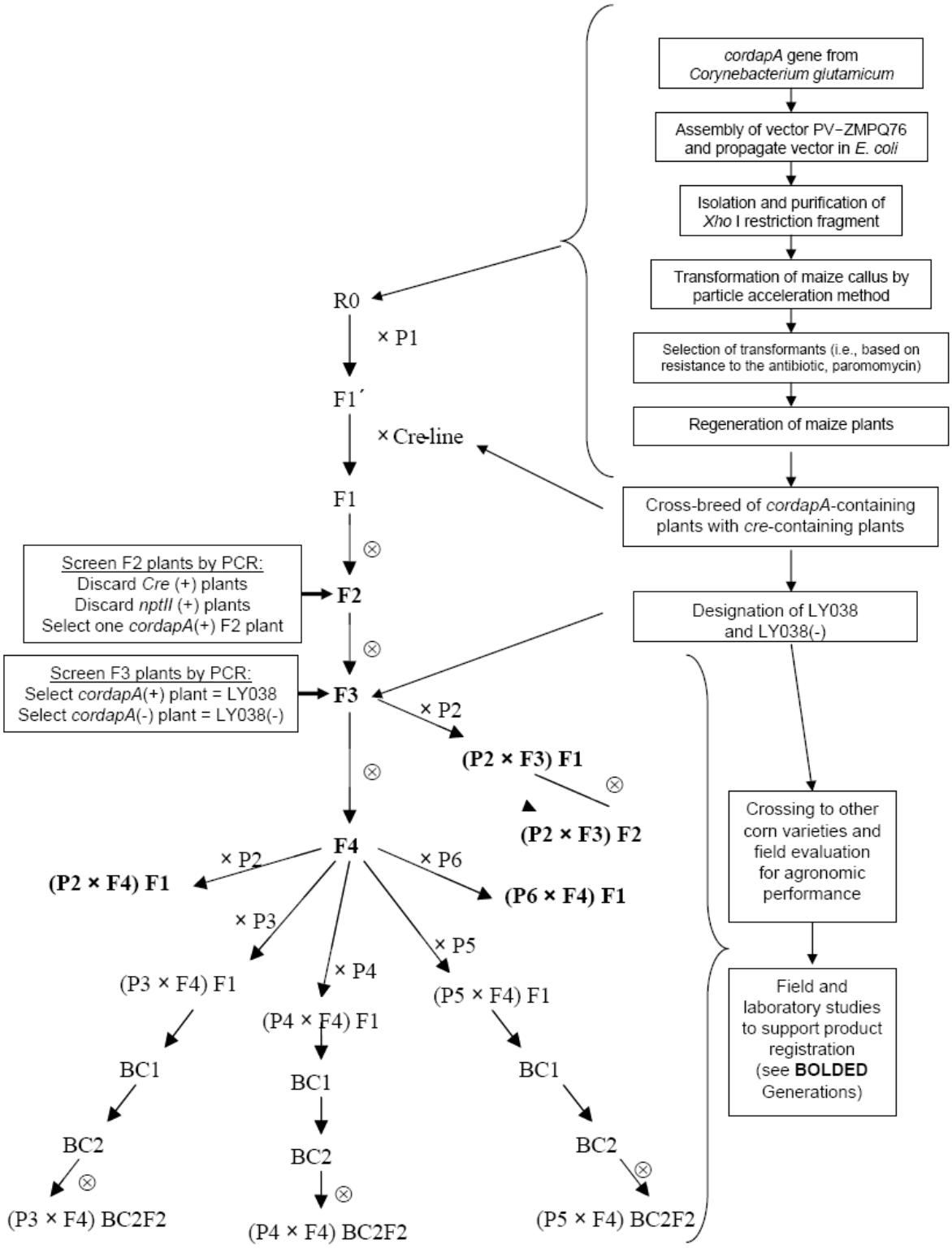

Figure 1 explanation:

The generation immediately prior to the one in which the *nptII* gene was excised by Cre-mediated recombination is designated F1'. Segregation analysis was performed on F1', (P2xF3)F2, (P3xF4)BC2F2, (P4xF4)BC2F2, and (P5xF4)BC2F2. Molecular generational stability analysis was performed on F2, F3, (P2xF3)F1, (P2xF3)F2, F4, (P2xF4)F1 and (P6xF4)F1 (all shown in bold font). Molecular characterisation was performed on (P2cF3)F2. Gene expression and compositional analyses were performed on (P2xF4)F2.
R0 = transformed plant
Pn = nontransgenic inbred line
Fn = filial generation
x contained in a circle = self-pollination
BCn = backcross generation.

3.2 Function and regulation of novel gene

cordapA

The *cordapA* gene is under the control of the *Zea mays globulin 1* (Glb1) promoter, which in wild-type maize directs expression of the most abundant embryo-specific protein in maize grain (Belanger and Kriz, 1991). The utilisation of the Glb1 promoter for *cordapA* transcription results in the expression of cDHDPS and the accumulation of lysine predominantly in the germ portion of the grain. Following the promoter is an intron sequence derived from the rice *actin-1* gene, the *rAct1 intron*, which enhances DNA transcription (McElroy *et al*., 1990). The *cordapA* gene is preceded by the *Zea mays* dihydrodipicolinate synthase choloplast transit peptide (mDHDPS CTP), to translocate cDHDPS to the plastid where the majority of amino acid biosynthesis occurs (Frisch *et al*., 1991). The 3' non-translated region of the globulin 1 gene follows the *cordapA* gene and contains the polyadenylation signal that directs the termination and maturation of the *cordapA* transcript (Belanger and Kriz 1991).

Table 1: Genetic elements in LY038 corn

Genetic element	Size (bp)	Function
Glb1 promoter	1392	The promoter from the *Globulin 1* (Glb1) gene from *Zea mays* (Belanger and Kriz 1991)
rAct1 intron	481	Intron from the rice *actin* gene (McElroy *et al.,* 1990)
mDHDPS CTP	171	The chloroplast targeting sequence from the maize DHDPS (Frisch *et al.,* 1991)
cordapA	903	The coding region of dihydrodipicolinate synthase (dapA) from *Corynebacterium glutamicum* in the lysine biosynthetic pathway, conferring resistance to lysine feedback inhibition (Bonnassie *et al*., 1990).
Glb1 3' UTR	1000	The 3' nontranslated region from the *Globulin 1* (Gbl1) gene from *Zea mays* which directs the polyadenylation of the mRNA (Belanger and Kriz 1991)

3.3 Characterisation of the genes in the plant

Studies submitted:
Groat JR, Wolff BJ, Scanlon NK and Masucci JD (2005) Molecular analysis of the LY038, LY038(-) Control and Inbred Maize Lines Contributing to the Genetic Background of LY038 and LY038(-), Monsanto Company, Study Id 05-01-72-04.

Mittanck D.W., Rice, J.F., Palmer, G.M. and Reiser, S.E. (2004) Amended Report for MSL-17770: Molecular Analyses of Lysine Maize LY038, Monsanto Company, Study Id 02-01-72-04.

Silvanovich, A and McCoy R. L. (2004) Bioinformatices Evaluation of DNA sequences Flanking the 5' and 3' Junctions of the Inserted DNA in Lysine Maize LY038: Assessment of putative polypeptides, Monsanto Company, Study Id 04-01-72-03

Insert and copy number

Southern blot analysis, using a number of restriction sites, a variety of probes and five control corn lines, indicated that only once copy of the *cordapA* gene and associated regulatory elements is present in the genome of LY038 at a single locus. The *nptII* and *cre* gene cassettes and plasmid backbone sequence were not detected by Southern blot analysis. No novel DNA was detected in the negative segregrant, LY038(-).

The arrangement of the insert in LY038 is shown in Figure 2. Arrows indicate the end of the insert and the beginning of the corn genomic DNA. Identified on the map are the genetic elements of the *cordapA* cassette and well as the locations of selected restriction enzymes.

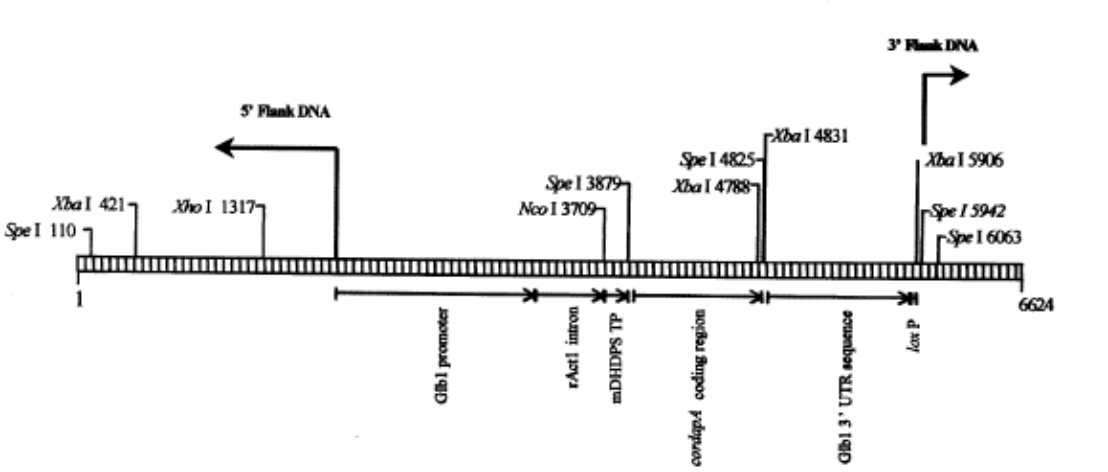

Figure 2: Linear map of the insert and adjacent DNA flanking the insert in LY038 corn

PCR and sequence analysis

The organisation of the elements within the LY038 corn insert was confirmed by PCR analysis of four overlapping regions of DNA that span the entire length of the insert and corn genomic flanking regions. Sequence analysis demonstrated that the sequence of the insert (4176 bp) is identical to that of the gene construct in the transforming plasmid, with the exception of two single base pair changes (both C to T) in the Glb1 promoter. There were no changes in the coding region of the inserted DNA. The recombined *lox*P site resulting from the removal of *nptII* indicates that the Cre-*lox* recombination occurred as expected.

Flanking regions and putative Open Reading Frame analysis

The flanking corn genomic DNA was also sequenced. 1781 bp and 667 bp were sequenced at the 5' and 3' ends of the insert respectively. Analysis of the sequence spanning the junction regions indicated that in the six reading frames at each junction, only one novel open reading frame starting with a methionine codon and of significant size (>100 amino acids) was identified. Bioinformatics analysis of this and the other 11 other putative open reading frames was performed and is discussed further in section 4.5.

Conclusion

Detailed molecular analyses have been performed on corn line LY038 to characterise the novel genes present in the genome. Results indicate that there is one insertion site containing one copy of the intact *cordapA* gene cassette.

Sequence analysis showed that two single nucleotide changes had occurred within the non-coding region of the insert (Glb1 promoter region). One novel ORF (>100 amino acids and starting with methionine) was created by the insertion.

3.4 Stability of the genetic changes

Inheritance of cordapA in LY038 corn

Heritability of the *cordapA* gene in LY038 corn was evaluated by determining segregation ratios in three generations: in the F1' generation prior to excision of the *nptII* marker gene; in the F3 generation which was obtained after marker excision; and in the F4 generation which had subsequently undergone two rounds of backcrossing to conventional inbred lines (see Figure 1 which gives the breeding history of LY038 corn). For analysis of the F3 generation, individual plants homozygous for the *cordapA* gene cassette were crossed to a conventional inbred line (P2), and progeny resulting from this cross were subsequently self-pollinated to yield (P2xF3)F2 progeny. For analysis of the F4 generation, plants homozygous for the *cordapA* gene cassette were crossed to each of three different conventional inbred lines (P3, P4 and P5) and then backcrossed to the corresponding inbred line for two subsequent generations while selecting for individual plants that carried the *cordapA* gene cassette. BC2 plants heterozygous for the *cordapA* gene cassette were subsequently self-pollinated to yield the BC2F2 generation.

The expected segregation ratio for the F1' generation is 1:1 (insert positive progeny: insert negative progeny), whereas the expected ratio for the subsequent generations, which were evaluated by analysing progeny obtained by self-pollinating heterozygous plants is 3:1. Positive plants in the F1' generation were identified by screening for the presence of the *nptII* gene product, as this gene was physically linked to the *cordapA* gene. For analysis of *cordapA* segregation in subsequent generations, *cordapA* specific oligonucleotides were used in standard DNA analytical procedures.

Overall five generations were examined. The results of the Chi square test are shown in Table 2. There was no significant difference between the observed and expected segregation ratios for LY038 corn. This indicates stable integration of the *cordapA* gene at a single site in the genome.

Table 2: Segregation frequency of the *cordapA* gene in LY038 corn

Generation	Observed positives	Observed negatives	Expected positives	Expected negatives	Chi square
(R0xP1)=F1'	49	44	46.50	46.50	0.17 ns
(P2xF3)F2	145	46	143.25	47.75	0.04 ns
(P3xF4)BC2F2	348	110	343.50	114.50	0.19 ns
(P4xF4)BC2F2	586	176	571.50	190.50	1.37 ns
(P5xF4)BC2F2	460	175	476.25	158.75	2.08 ns

ns = non significant

Stability of the insert

Southern blot analyses with a variety of probes and restriction enzymes were performed to demonstrate the stability of the insert over multiple generations of LY038 corn [including F2, F3, (P2xF3)F1, (P2xF3)F2, F4, (P2cF4)F1 and (P6xF4)f1].

These results were consistent with previous molecular characterisation results and established the stability of the inserted DNA over multiple generations representing each branch point of the breeding tree and confirmed the absence of both the *nptII* and *cre* gene cassettes.

3.5 Antibiotic resistance genes

The *nptII* gene was removed by Cre-*lox* recombination, therefore no antibiotic resistance genes are present.

4. CHARACTERISATION OF NOVEL PROTEINS

4.1 Biochemical function and phenotypic effects

Lysine synthesis

Humans and other monogastric animals cannot synthesise 9 of the 20 common amino acids found in proteins and therefore these essential amino acids need to be obtained from the diet. Lysine is an essential amino acid and is important because it is one of the most limiting amino acids in cereal grains (which represent the largest source of food and feed worldwide). The lysine content of most major crop plants is limited due to feedback inhibition by free lysine of a vital enzyme in the lysine biosynthesis pathway (dihydrodipicolinate synthase, DHDPS). The limited amount of natural lysine in cereal based animal feeds means that these feeds often must be supplemented with lysine. The requirement for protein supplementation can be costly and inefficient for animal nutrition. For this reason increasing lysine levels in grain has been a primary objective of plant breeding since the 1960s (MERTZ *et al*., 1964). Because of its nutritional importance, the regulation of lysine metabolism has been studied extensively at the biochemical, genetic and molecular levels in a wide range of organisms such as bacteria, plants and mammals.

In plants and some bacteria, lysine is synthesised via the aspartate pathway that also leads to the synthesis of methionine and threonine (Figure 3).

The enzyme DHDPS catalyses the first committed step specific to lysine biosynthesis (Galili, 1995). As this pathway has been modified in LY038 grain, the levels of other products of this pathway may be altered, and therefore in addition to the usual compositional analyses (including amino acids), levels of homoserine and 2,6-diaminopimelic acid were analysed and compared with conventional corn grain. 2,6-diaminopimelic acid is the penultimate metabolite in lysine biosynthesis; the other intermediary metabolites in the pathway are potentially unstable or known to be present in very low levels in plant species. This analysis is discussed in Section 5.

The possibility that increased synthesis of lysine may lead to reduced levels of methionine and threonine was also considered. Amino acid analyses presented in Section 5 indicate that the levels of these two amino acids in LY038 corn are present at levels normally found in corn.

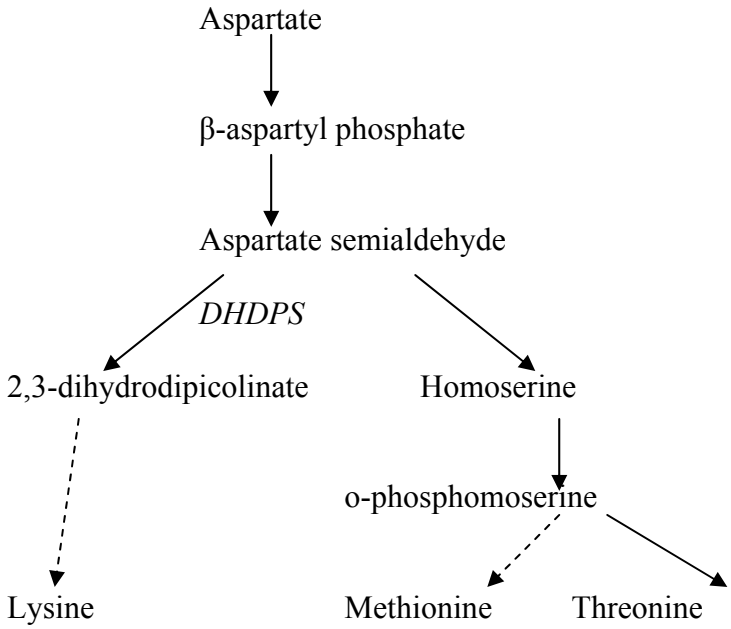

Figure 3: Aspartate pathway of lysine biosynthesis

cDHDPS

LY038 corn has been modified to produce a bacterial DHDPS protein (from *C. glutamicum)*. This protein is 304 amino acids in length and approximately 33 kDa. The bacterial DHDPS protein is referred to as cDHDPS and belongs to the family of related DapA (DHDPS) proteins. DHDPS proteins isolated from a number of species including spinach, pea, maize, *E. coli* and *Bacillus subtilis* have been extensively characterised (Wallsgrove and Mazelis, 1981; Frisch *et al.,* 1991; Dereppe *et al*., 1992; Karsten, 1997).

The mechanism for action of cDHDPS has been well characterised (Karsten, 1997). DHDPS mediates a critical rate-limiting step in the lysine biosynthetic pathway that is controlled by lysine feedback inhibition. The enzyme catalyses the condensation of L-aspartate-4-semialdehyde and pyruvate to form 2,3-dihydropicolinate that is converted to lysine though a series of subsequent enzymatic reactions (Figure 3). In contrast to the native maize DHDPS, the variant of this enzyme from *C. glutamicum* is not sensitive to lysine feedback inhibition. This leads to a build up of lysine, seen as an increase in free lysine (or non-protein incorporated lysine) levels.

Lysine catabolism

In plants and animals, lysine is primarily catabolised via the α-aminoadipic acid pathway by two linked enzymes, lysine-ketoglutarate reductase (LKR) and saccharopine dehydrogenase (SDH), to produce α-aminoadipic acid or pipecolic acid (Figure 4). LKR and SDH enzyme activity are closely linked as they are both present on the same protein (Kemper *et al*., 1998; Papes *et al*., 1999). LKR condenses lysine with α-ketoglutarate to produce saccharopine. Saccharopine is subsequently metabolised by SDH to α-aminoadipic semialdehyde to yield glutamate and α -ketoadipic acid, and ultimately enters the citric acid cycle as acetoactyle-CoA (Devlin, 2001). In some plant species, lysine can be decarboxylated to the metabolite cadaverine through the action of the enzyme lysine decarboxylase.

As LY038 grain is expected to contain higher levels of free lysine than conventional grain, it was anticipated that it might also contain higher level of lysine catabolites. For this reason, the levels of cadaverine, saccharopine, α-aminoadipic acid, and pipecolic acid in LY038 grain were examined. This is discussed in Section 5 as part of the compositional analysis.

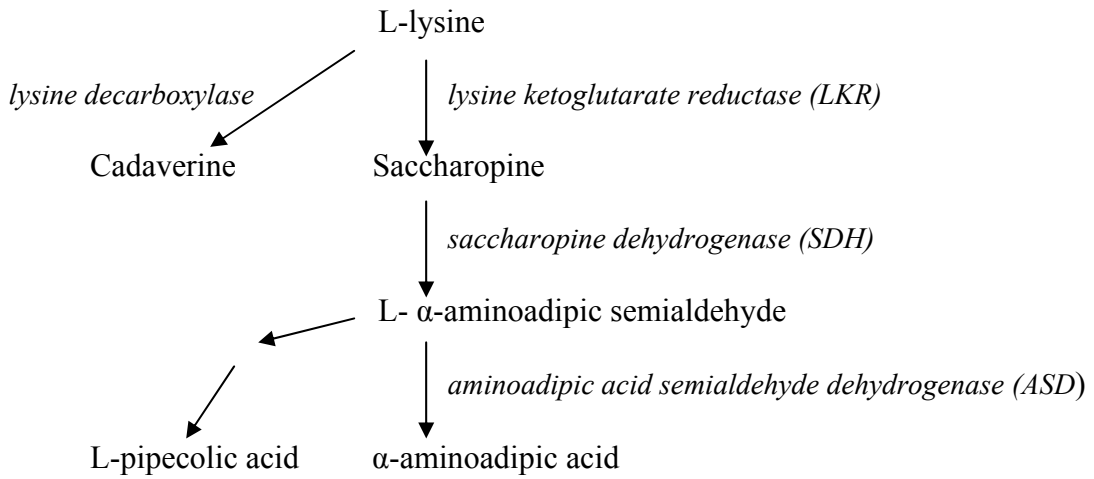

Figure 4: Enzymatic catabolism of lysine in seed (adapted from Galili, 1995)

Toxicity of lysine

A review of the nature of hazards associated with excessive intake of amino acids was conducted in 2004 and published in the Journal of Nutrition (Garlick, 2004). The studies to date show that lysine has little toxicity. It is used as a treatment for cold sores in doses around 3g/day. Adults given 40 g lysine hydrochloride/day for 2-5 days or up to 3 g/day for up to 6 months showed no adverse effects except upset stomach. No ill effects were reported in children (aged 10 – 14 years) injected with 14 – 22 g lysine hydrochloride, in infants (aged 4-11 months) supplemented with up to 1 g lysine/8 oz of milk in increments over 3-4 days or in infants (aged 1-5 months) given a dose of 220 mg lysine/kg (Garlick 2004).

Lysine levels in other commonly consumed foods are significantly higher than those levels in LY038 and are shown in Table 3. It can be seen that LY038 grain has approximately 160 mg / 100 gm more lysine than the control corn grain, however when compared to lysine from other dietary sources this is not a large amount of lysine and does not represent a human health concern.

Further, it is expected that the amount of LY038 grain entering the food supply will be small, and that many food products derived from corn, such as high fructose corn syrup would not be expected to contain any lysine.

Table 3: Lysine levels in some commonly consumed foods

Food	Lysine content (ppm in the food)[1]
LY038 grain	4800
Control corn grain	3200
Egg (hard boiled)	9640
Fish	15,000 – 22,000
Red meat (beef & lamb)	15,000 – 33,000
Chicken	17,000 – 27,000
Cheese	7,000 – 28,000
Lentils	4,890
Rolled oats	4,430
Broccoli	2,470

[1] Values are from (ANZFA, 1999) except for those for LY038 grain and control corn grain, which are from Appendix IV, page 224, of Monsanto's application to FSANZ and expressed on a dry weight basis. Values have been converted from mg/g or mg/100 g of food to ppm.

4.2 Protein expression analysis

Characterisation of the novel protein as expressed in LY038

The cDHDPS protein produced in LY038 corn was characterised to determine that the expected protein was being produced. The *cordapA* gene encodes a 32 kDa protein (calculated based on predicted amino acid sequence) consisting of a single polypeptide of 303 amino acids (including three amino acids from the maize chloroplast transit peptide). The molecular identity and biochemical characteristics of the cDHDPS protein expressed *in planta* were examined using a variety of biochemical techniques.

SDS-PAGE and Western blot analysis of the LY038 produced cDHDPS protein revealed a protein with a molecular weight of approximately 33 kDa. This band was excised and N-terminal sequence analysis performed, which confirmed the presence of two N-terminal sequences in this band, both of which were consistent with the expected sequence of the cDHDPS protein. One sequence commenced with the three amino acids of the chloroplast transit peptide, the other commenced at the N-terminus of the cDHDPS protein. This observation is not uncommon in plant-produced proteins as the cleavage of the chloroplast transit peptide can involve processing at different sites. The 12 N-terminal amino acids of the cDHDPS protein were shown to be present.

MALDI-TOF mass spectrometry analysis identified 17 protein fragments that matched the expected mass of the trysin-digested cDHDPS protein. These covered over 58% of the protein and identified it as the expected protein. A protein can usually be identified when 40% of the mass fragments are identified from the analysed protein. Immunoblot analysis with cDHDPS specific antisera (goat antisera) also positively identified the approximately 33 kDa band as cDHDPS.

Glycosylation analysis indicated that the LY038 produced cDHDPS is not glycosylated and in an enzyme assay it was determined that the specific activity of this enzyme is 68 ± 3 U/mg total protein.

Expression of the novel protein in LY038

Study submitted:
Hartmann, A.J., Bhakta, N.S., Bookout, J.T., Jennings, J.C. (2004) Assessment of cDHDPS Protein Levels in Maize Tissues from Lysine Maize LY038 collected from 2002 US Field Trials. MSL-19262.

cDHDPS expression is under the control of the *Glb* promoter and is therefore expected to be expressed primarily in the grain. Expression levels were determined using an enzyme-linked immunosorbent assay (ELISA). Tissue samples were collected from LY038 corn grown at five field sites in the US during the 2002 growing season. cDHDPS levels for all tissue types were determined on a microgram per gram fresh weight (fwt) basis. Moisture content was then measured for all tissue types and protein levels were converted to a dry weight (dwt) basis. The results are shown in Table 4.

The mean cDHDPS protein levels across the five sites in grain, forage, whole plant, forage root, root and pollen tissues were 26, 0.94, 0.081, 0.069, 1.5 and 0.78 µg/g dry weight respectively. Levels of cDHDPS in LY038 corn leaf tissues harvested at four time points throughout the growing season (over season leaf 1-4) were less than the assay limit of detection for leaf tissue (LOD 0.013 µg/g fresh weight).

These results confirm that cDHDPS expression occurs primarily in the LY038 corn grain, however, low levels of expression occur throughout the plant.

Table 4: Summary of cDHDPS levels in LY038 corn

Tissue type[1]	Mean cDHDPS µg/g fwt[1] (SD)	Range[2] (µg/g fwt)	Mean cDHDPS µg/g dwt[3] (SD)	Range (µg/g dwt)	LOQ/LOD (µg/g fwt)
Grain	24 (9.1)	13 – 45	26 (10)	14 – 49	0.044/0.021
Forage	0.25 (0.21)	0.034 – 0.79	0.94 (0.75)	0.15 – 2.8	0.0025/0.00056
Whole plant	0.0093 (0.0083)	0.0026 – 0.019	0.080 (0.068)	0.024 – 0.22	0.0025/0.00056
Forage root	0.010 (0.0043)	0.0052 – 0.019	0.069 (0.031)	0.031 – 0.11	0.0050/0.0050
Root	0.14 (0.23)	0.011 – 0.62	1.5 (2.2)	0.099 – 6.2	0.0050/0.0050[3]
Pollen	0.43 (0.14)	0.27 – 0.67	0.78 (0.24)	0.45 – 1.1	0.025/0.0052
Over season Leaf 1-4	<LOD	-	n/a[4]	-	0.038/0.013

[1] The number of samples used for data analysis (n) is as follows:
Grain, forage, forage root, pollen and over season leaf n = 15
Whole plant n = 16
Root n = 12
[2] Range across sites
[3] The LOQ and LOD for cDHDPS in root tissues are identical
[4] Protein levels that were <LOD on a fwt basis were not converted to dwt values

4.3 Potential toxicity of novel proteins

Proteins which cause toxicity act via acute mechanisms and generally at very low doses (Sjoblad *et al.*, 1992). Therefore, when a protein demonstrates no acute oral toxicity at a high dose level using a standard laboratory mammalian test species, this supports the determination that the protein will be non-toxic to humans and other mammals, and will not present a hazard under any realistic exposure scenario, including long-term exposures.

Studies submitted:
Kaempfe, A.J. (2003) An Acute Oral Toxicity Study in Mice with *E. coli*-produced cDHDPS Protein. MSL-18735, and unpublished study by Monsanto Company

Rice, E.A., Kapadia, S.A., Dalton, C.M., Brown, T.P., Thoma. R.S., Hileman, R.E. and Astwook, J.D. (2003) Characterisation of the *E. coli*-Produced *Corynebacterium glutamicum* Dihydrodipicolinate Synthase (cDHDPS) Protein. MSL-18365, an unpublished study by Monsanto Company

Rice, E.A., Kapadia, S.A., Thoma, R.S. and Hileman, R.E. (2003) Characterisation of the cDHDPS Protein Purified from Grain of Lysine Maize LY038 and Assessment of the Physicochemical and Functional Equivalence of the Plant-Produced cDHDPS Protein and the *E. coli*-produced cDHDPS Protein. MSL-18585, an unpublished study by Monsanto Company

The Applicant submitted an acute oral toxicity study using mice to support the safety of cDHDPS. As it is difficult to extract and purify sufficient quantities of the subject protein from transgenic corn plants for the acute oral toxicity studies, it is standard practice to instead use an equivalent protein that has been produced using a bacterial expression system. Prior to use, the bacterially produced protein must be are compared to the novel protein produced in the plant in order to establish their equivalence. The cDHDPS protein used in the toxicity study was produced in recombinant *E. coli* and therefore the molecular identity and biochemical characteristics of this protein expressed in the bacterial-expression system were examined.

This involved a number of biochemical technical (SDS-PAGE and Western blotting, N-terminal sequencing, immunoreactivity, glycosylation analysis, peptide mass fingerprinting, MALDI-TOF mass spectrometry and enzyme activity).

These studies allowed the *E. coli* produced cDHDPS to be compared with the LY038 produced cDHDPS and established that these two proteins are equivalent. Therefore the *E. coli* produced cDHDPS was used in the toxicity studies.

Test material	*E. coli* produced cDHDPS
Vehicle	Sodium phosphate buffer
Test Species	10 male and 10 female CD-1 per test group
Dose	800 mg/kg body weight in one gavage dose of 33 mL/kg body weight
Control	bovine serum albumin (BSA)

The mice received a single dose of either 800 mg/kg body weight cDHDPS or BSA and were observed for two weeks. Parameters evaluated included body weights and detailed clinical observations. All animals were observed for gross pathological changes.

All mice appeared normal and there were no mortalities during the course of the two week study. There were no significant differences in body weight or body weight changes between the test and control groups.

At the end of two weeks, all animals were killed and subjected to further analysis. Gross pathological findings included abnormal content of the digestive system (one test male), a diverticulum of the jejunum (one control female) and a lesion on the kidney (one control female and one test female). These findings were not considered to be treatment related. Two test females exhibited periovarian cysts, however this is a common finding in female mice of this strain and was not considered to be treatment related.

Therefore, under the conditions of this study, the acute oral LD_{50} of cDHDPS in mice is greater than 800 mg/kg bw.

Similarity of cDHDPS with known protein toxins

Study Submitted:
McCoy, R.L and Silvanovich, A. (2003) Bioinformatics Analysis of the cDHDPS Protein Expressed in Lysine Maize Event LY038 and LY049 Utilising the AD4, TOXIN5 and ALLPEPTIDES Databases. MSL-18744, an unpublished study conducted by Monsanto Company

Bioinformatic analyses were done to assess the cDHDPS for any similarity with known protein toxins. The similarity search was conducted against the ALLPEPTIDES (protein sequence database comprised of GenBank and SwissProt) and TOXIN5 (toxin sequence protein database) databases. Potential structural similarities were evaluated using the FASTA sequence alignment tool.

The most significant alignment identified from the ALLPEPTIDES database was to the *C. glutamicum* DHDPS, demonstrating 100% identity over a 300 amino acid window. The remaining 450 alignments with significant E scores were between cDHDP and members of the N-acetylneuraminate lyase subfamily of pyruvate-dependent class I aldolases (Lawrence *et al*., 1997) found in a number of organisms including bacteria, rodents and humans. This is not surprising as the DHDPS enzyme is a member of this subfamily, which does not pose any likely risk of adverse biological activity to humans or animals.

The most significant alignment identified from the TOXIN5 database was the *E. coli* N-acetylneuraminate lyase protein (accession no. BAB37521), demonstrating 29% identity over a 290 amino acid window and an E score of 2.9 e-18.

As mentioned above, DHDPS is part of this family of proteins so similarity with this protein is not surprising. However, the *E. coli* N-acetylneuraminate lyase protein is only inadvertently part of the toxin database, which is an uncurated collection of publicly available sequence datasets that have annotations that include the word ‘toxin’. In this case, the publication title included the words ‘verotoxin 2 genes’ that are unrelated to the N-acetylneuraminate lyase protein (Makino *et al*., 1999). Inspection of other alignments did not show any significant similarities between the cDHDPS amino acid sequence and any other proteins in the toxin database.

Conclusion

The data from acute oral toxicity studies and bioinformatics analyses of the novel protein indicate that it is neither toxic at high levels in mice nor has any similarity with known protein toxins. cDHDPS is highly similar to other DHDPS proteins found in a wide range of organisms.

4.4 Potential allergenicity of novel proteins

When assessing a new GM food, a concern is that new proteins introduced into food may cause allergic reactions in some individuals. The potential allergenicity of a novel protein is evaluated using an integrated, step-wise, case-by-case approach relying on various criteria used in combination, since no single criterion is sufficiently predictive of either allergenicity or non-allergenicity. The assessment focuses on the source of the novel protein, any significant amino acid similarity between the novel protein and that of known allergens, and the structural properties of the novel protein, including susceptibility to degradation in simulated digestion models. Applying such criteria systematically provides reasonable evidence about the potential of the newly introduced protein to act as an allergen (Jones and Maryanski, 1991; Lehrer and Reese, 1998). The potential allergenicity of cDHDPS was assessed using these criteria.

Source of the protein

The novel protein cDHDPS is derived from *C. glutamicum*, a non-pathogenic bacteria that is widely distributed in nature. *C. glutamicum* is not a known source of allergens.

Similarity to known allergens

Study submitted:
McCoy, R.L and Silvanovich, A. (2003) Bioinformatics Analysis of the cDHDPS Protein Expressed in Lysine Maize Event LY038 and LY049 Utilising the AD4, TOXIN5 and ALLPEPTIDES Databases. MSL-18744, an unpublished study conducted by Monsanto Company

Potential structural similarities between cDHDPS and the proteins in the allergen database (AD4 – comprises allergen, gliadin and glutinin sequences) were evaluated using the FASTA sequence alignment tool. Identified proteins were ranked according to their degree of similarity. The most significant alignment was to the *Mercurialis annua* profilin allergen, demonstrating 23.9% identity over a 92 amino acid window and an *E* score of 1.4. The length of the overlap is relatively short when compared to the full length (303 amino acids) of cDHDPS. Moreover, the longest stretch of the contiguous amino acid identities consists of only three amino acids. Consequently no structural or functional homology between these two proteins can be inferred.

Furthermore, no immunologically relevant sequences (eight contiguous amino acid identities) were detected when the cDHDPS sequence was compared to the AD4 sequence database. A sequence length of eight amino acids was chosen as the appropriate length on which to conduct this search, as when shorter sequences are used (e.g. 7 or 6 amino acid sequences) a large number of positive false results can be found.

These data demonstrate that cDHDPS is unlikely to share structurally or immunologically relevant sequence similarities with known protein allergens.

In vitro digestibility

Studies submitted
Rice, E.A., Kapadia, S.A. and Hileman, R.E. (2003) Assessment of the *in vitro* Digestibility of the cDHDPS Protein in Simulated Gastric Fluid. MSL-18676, un unpublished study conducted by Monsanto Company

Typically, most food allergens tend to be stable to the peptic and acidic conditions of the digestive system if they are to reach and pass through the intestinal mucosa to elicit an allergic response (Astwood and Fuchs, 1996; Metcalfe *et al*., 1996; Kimber *et al*., 1999). The *in vitro* digestibility of cDHDPS was therefore assessed in simulated gastric fluid (SGF) contain the proteolytic enzyme pepsin. *E. coli* produced cDHDPS was used in this assay.

cDHDPS was rapidly digested after incubation in SGF. Greater than 96% and 98% of the cDHDPS enzyme was observed to be digested within 30 seconds when analysed using Colloidal Brilliant Blue stained polyacrylamide gels and Western blot techniques respectively. No cDHDPS was detectable at the second time point (2 minutes) by either method. No cDHDPS fragments were visible by either method at any of the eight time points.

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

Studies Submitted:
McCoy. R.L. and Silvanovich, A. (2004) Bioinformatics Evaluation of the cDHDPS Protein Coding Sequence in Lysine Maize LY038: Assessment of Putative Polypeptides. MSL 19181. An unpublished study by Monsanto Company
McCoy. R.L. and Silvanovich, A. (2004) Bioinformatics Evaluation of DNA Sequences Flanking the 5' and 3' Junctions of the Inserted DNA in Lysine Maize LY038: Assessment of Putative Polypeptides. MSL 19182. An unpublished study by Monsanto Company

As part of a comprehensive safety assessment, bioinformatics analyses were performed to assess the similarity to known allergens, protein toxins or pharmacologically active proteins of the putative polypeptides encoded by the DNA spanning the junctions between corn genomic DNA and the 5' and 3' ends of the inserted DNA. Sequences spanning either the 5' or 3' junction region were translated from stop codon to stop codon in all six reading frames. As mentioned in Section 3.3, only one novel open reading frame starting with a 'start' codon and of significant size (>100 amino acids) was identified. However, bioinformatics analysis of this and the other 11 other putative open reading frames was performed using the ALLPEPTIDES, TOXIN5 and AD4 (the allergen database) databases. Analysis was also done on the putative polypeptides encoded by reading frames two to six of the cDHDPS protein coding sequence of the insert, however due to the lack of appropriate regulatory elements, it is unlikely that these putative polypeptides are transcribed.

No evidence exists to suggest that any of these ORFs might be transcribed. No biologically relevant structural similarities to allergens, toxins or pharmacologically active proteins were observed for any of the putative polypeptides. Furthermore, no short (eight amino acids) polypeptide matches were shared between any of the putative polypeptides and the allergens in the databases. These data demonstrate the lack of structurally relevant similarities to toxins or other pharmacologically active proteins for all of the putative polypeptides analysed.

The results of these analyses indicate that in the unlikely event that any of these putative polypeptides were to be transcribed, and that if a transcript were to be translated, the translation product would not share sequence similarity or identity to any known allergens, protein toxins, or pharmacologically active proteins.

4.6 Conclusion regarding characterisation of the novel protein

Corn line LY038 expresses one novel protein, cDHDPS, predominantly in the corn grain (13-45 µg/g fresh weight), but also at low levels in other plant tissues.

The novel protein has been well characterised in LY038 corn: it is 303 amino acids in length and has a molecular weight of approximately 33 kDa. Various biochemical analyses have shown that the desired protein is expressed.

A number of studies have demonstrated that cDHDPS has limited potential to be toxic or allergenic; the source of this protein is not known to be toxic or allergenic, the protein has no similarity to known protein allergens or toxins, it has low acute oral toxicity and is rapidly digested.

5. COMPARATIVE ANALYSES

Most crops, including oilseed crops, exhibit considerable variability in their nutrient composition. Environmental factors and the genotype of the plant have an enormous impact on composition. Thus, variation in these nutrient parameters is a natural phenomenon and is considered to be normal.

A comparative approach focussing on the determination of similarities and differences between the GM food and its conventional counterpart aids in the identification of potential safety and nutritional issues and is considered the most appropriate strategy for the safety and nutritional assessment of GM foods (WHO, 2000). The critical components to be measured are determined by identifying key nutrients, key toxicants and antinutrients for the food source in question (FAO, 1996). The key nutrients and toxicants/antinutrients are those components in a particular food that may have a substantial impact in the overall diet. These may be major constituents (e.g., fats, proteins, carbohydrates) or minor components (e.g., minerals, vitamins). Key toxicants are those toxicologically significant compounds known to be inherently present in the plant, such as those compounds whose toxic potency and level may be significant to health (e.g., solanine in potatoes if the level is increased).

5.1 Nutrient analysis

Study submitted

Reynolds, T. Nemeth, M., Fuhrman, J., Trujillo, W. and Sorbet, R. (2004) Compositional analysis of forage and grain collected from lysine maize LY038 and LY038 x MON810 grown in U.S. field trials in 2002. MSL-18881. An unpublished study by Monsanto Company

LY038 corn and LY038(-) corn were grown at five replicated field sites in Illinois, Iowa and Nebraska in the United States during the 2002 growing season. Four commercially available maize hybrids were grown at each of the five field sites to provide a total of 20 reference substances (18 unique reference materials).

Forage and grain samples were collected from all plots and analysed for nutritional components, antinutrients and secondary metabolites consistent with the international consensus recommendations for compositional evaluation of maize (OECD, 2002). Free lysine (non-protein incorporated lysine) was determined in addition to total lysine. Homoserine and 2,6-diaminopimelic acid (components of the lysine synthesis pathway) and cadaverine, saccharopine, α-aminoadipic acid and pipecolic acid (possible end products of lysine catabolism) were also analysed.

Compositional analyses of the grain samples included proximates, fibre, amino acids, free lysine, fatty acids, vitamins, minerals, carbohydrates, antinutrients (phytic acid and raffinose), secondary maize metabolites as determined by the OECD (furfural, ferulic acid, and p-coumaric acid) and the additional lysine related metabolites mentioned above. In all, 75 different components were analysed in LY038 corn grain. However, more than half the measurements for sodium and 14 of the fatty acids were below the limit of quantitation for the test, control and reference grains and were excluded from the statistical analysis. Furfural, cadaverine and 2,6-diaminopimelic acid were below the limit of quantitation for all the grain samples analysed (LY038, LY038(-) and reference samples) so no values for these analytes are reported in the tables of compositional results. Values for the lysine catabolite, α-aminoadipic acid, were below the limit of quantitation (5 ppm) in the control and reference grain, therefore the values for LY038 grain are summarised separately in Table 11.

Statistical assessment of the compositional data was conducted using a mixed model analysis of variance on six sets of comparisons including within site comparisons for each of the five sites and a sixth comparison across sites, referred to as the combined sites. Statistically significant differences were determined at the 5% level ($p < 0.05$). There were 336 statistical assessment conducted for the test grain compared to the control grain (six sets of analyses x 56 components assessed). Using the data for each component obtained from the 20 reference materials, a 99% tolerance interval (T.I.) was calculated to contain with 95% confidence, 99% of the values in the population of conventional maize. For any statistically significant difference between the LY038 grain and LY038(-) grain, the range of LY038 grain values was compared to the 99% tolerance interval. Significant differences that were not within the 99% T.I. were compared with historical and literature ranges for corn grain. Summaries of the results of the combined site analyses for all the components are shown in Tables 5 to 9.

Of the 336 statistical comparisons made between LY038 grain and the control grain, all but 22 were either not statistically significant differences or the LY038 range was within the 99% tolerance interval for the population of conventional maize. These 22 analyses are shown in Table 10. Fourteen of these 22 statistically significant differences were for lysine, free lysine and the lysine catabolite, saccharopine. The remaining eight differences were for copper, fat, fibre and folic acid. However, the values for copper, fat and folic acid were within the literature range and historical range for corn and therefore do not pose any food safety or nutritional concerns. The mean and range for dietary fibre was outside of the literature and historical range at one site, however, the difference between the test range and the upper 99% T.I. was very small (approximately 0.6% dwt). At the combined site, the mean was within the 99% T.I. Furthermore, the other four sites showed no significant difference between the test and control grain in terms of dietary fibre content.

The results of the saccharopine and α-aminoadipic acid analyses are discussed below.

Table 5: Summary of proximate and fibre analysis of LY038 grain (combined sites)

Component[1]	LY038 mean ± SE[2] (Range)	LY038(-) mean ± SE (Range)	p-value	Reference Range 99% TI[3]
Ash	1.44 ± 0.033 (1.19 – 1.73)	1.44 ± 0.033 (1.29 – 1.73)	0.974	(1.05 – 1.75) [0.92, 1.84]
Available carbohydrates	81.80 ± 0.62 (80.24 – 84.13)	82.02 ± 0.62 (80.34 – 84.75)	0.375	(80.26 – 87.96) [78.12, 92.06]
Moisture	8.91 ± 0.40 (7.47 – 10.50)	9.40 ± 0.40 (8.48 – 11.30)	0.74	(7.68 – 11.10) [6.32, 11.00]
Protein	12.90 ± 0.56 (11.44 – 14.48)	12.12 ± 0.56 (9.59 – 13.79)	0.002	(7.61 – 14.69) [3.86, 17.17]
Total fat	3.86 ± 0.20 (3.00 – 4.72)	4.42 ± 0.20 (4.00 – 5.16)	<0.001	(2.03 – 4.53) [1.36, 4.67]
Total dietary fibre	20.77 ± 2.48 (11.90 – 39.65)	15.99 ± 2.48 (10.96 – 21.30)	0.042	(12.58 – 35.31) [3.77, 39.08]
Acid detergent fibre	6.57 ± 0.42 (4.66 – 11.31)	5.80 ± 0.42 (4.20 – 6.82)	0.083	(4.29 – 9.56) [2.64, 10.00]
Neutral detergent fibre	12.56 ± 1.08 (8.01 – 18.28)	10.19 ± 10.8 (7.89 – 13.03)	0.025	(9.93 – 20.57) [5.82, 21.51]

[1] Shown as % dry weight, except for moisture which is % fresh weight
[2] Mean ± SE = least square mean ± standard error of the mean.
[3] The reference range is the range of values for the conventional corn varieties grown at the same 5 field sites. TI = tolerance interval specified to contain with 95% confidence, 99% of the population of conventional maize. Negative limits set to 0.

Table 6: Summary of amino acid levels in LY038 grain (combined sites)

Component (% total amino acids)	LY038 mean ± SE[1] (Range)	LY038(-) mean ± SE (Range)	p-value	Reference Range 99% TI[2]
Alanine	7.81 ± 0.065 (7.62 – 8.03)	7.88 ± 0.065 (7.49 – 8.05)	0.122	(7.22 – 8.33) [6.90, 8.67]
Arginine	4.26 ± 0.10 (3.79 – 4.73)	4.32 ± 0.10 (3.84 – 4.77)	0.491	(3.88 – 6.00) [3.32, 6.04)
Aspartic acid	6.20 ± 0.048 (5.89 – 6.44)	6.24 ± 0.048 (5.87 – 6.53)	0.523	(5.84 – 7.13) [5.86, 7.16]
Cysteine	2.03 ± 0.053 (1.90 – 2.13)	2.07 ± 0.053 (1.90 – 2.33)	0.344	(1.76 – 2.55) [1.48, 2.80]
Glutamic acid	19.98 ± 0.21 (19.14 – 20.55)	20.35 ± 0.21 (19.22 – 20.96)	0.002	(18.02 – 21.86) [16.76, 22.36)
Glycine	3.43 ± 0.081 (3.22 – 3.86)	3.51 ± 0.081 (3.16 – 3.99)	0.125	(3.27 – 4.61) [2.65, 4.98]
Histidine	2.76 ± 0.040 (2.63 – 2.89)	2.88 ± 0.04 (2.68 – 3.06)	<0.001	(2.63 – 3.39) [2.32, 3.64]
Isoleucine	3.41 ± 0.43 (3.21 – 3.54)	3.52 ± 0.043 (3.32 – 3.76)	0.014	(3.24 – 3.92) [3.13, 3.87]
Leucine	13.53 ± 0.19 (12.76 – 14.19)	13.64 ± 0.19 (12.63 – 14.48)	0.42	(11.13 ± 14.35) [10.15, 15.62]
Lysine (% of total amino acids)	3.81 ± 0.14 (3.08 – 4.50)	2.70 ± 0.14 (2.14 – 3.23)	<0.001	(2.38 – 4.07) [1.85, 4.29]
Methionine	2.13 ± 0.046 (1.88 – 2.40)	2.05 ±0.046 (1.85 – 2.37)	0.193	(1.54 – 2.41) [1.47, 2.46]
Phenylalanine	5.14 ± 0.048 (4.97 – 5.25)	5.22 ± 0.048 (4.86 – 5.41)	0.009	(4.67 – 5.43) [4.49, 5.68]
Proline	8.87 ± 0.10 (8.04 - 9.34)	9.08 ± 0.10 (8.38 – 9.40)	0.058	(7.92 – 10.18) [7.89, 10.23]
Serine	5.06 ± 0.054 (4.84 – 5.32)	5.11 ± 0.054 (4.90 – 5.37)	0.186	(4.79 – 5.55) [4.73, 5.60]
Threonine	3.11 ± 0.039 (2.91 – 3.26)	3.20 ± 0.039 (2.93 – 3.46)	0.082	(2.84 – 3.62) [2.73, 3.82]
Tryptophan	0.52 ± 0.024 (0.40 – 0.64)	0.55 ± 0.024 (0.43 – 0.72)	0.09	(0.45 – 0.90) [0.29, 0.89]
Tyrosine	3.34 ± 0.18 (2.26 – 3.85)	3.02 ± 0.18 (2.17 – 4.68)	0.234	(1.83 – 3.82) [2.04, 4.17]
Valine	4.62 ± 0.051 (4.37 – 4.85)	4.65 ± 0.051 (4.41 – 4.87)	0.509	(4.42 – 5.22) [4.15, 5.51]

[1] Mean ± SE = least square mean ± standard error of the mean.
[2] The reference range is the range of values for the conventional corn varieties grown at the same 5 field sites. TI = tolerance interval specified to contain with 95% confidence, 99% of the population of conventional maize. Negative limits set to 0.

Table 7: Summary of fatty acid levels in LY038 grain (combined sites)

Component[1] (% total fatty acids)	LY038 mean ± SE[2] (Range)	LY038(-) mean ± SE (Range)	p-value	Reference Range 99% TI[3]
Palmitic	10.86 ± 0.061 (10.58 – 11.83)	10.96 ± 0.061 (10.78 – 11.25)	0.184	(9.27 – 13.15) [7.42, 15.14]
Stearic	2.26 ± 0.031 (2.06 – 2.42)	2.20 ± 0.031 (2.12 – 2.27)	0.127	(1.65 – 2.42) [1.26, 2.67]
Oleic	31.81 ± 0.41 (30.62 – 33.39)	30.59 ± 0.41 (29.08 – 31.49)	<0.001	(21.44 – 35.65) [9.97, 43.10]
Linoleic	53.24 ± 0.40 (51.77 – 54.41)	54.48 ± 0.40 (53.61 – 0.97)	<0.001	(50.16 ± 64.33) [42.12, 74.18]
Linolenic	0.96 ± 0.017 (0.89 – 1.02)	0.91 ± 0.017 (0.86 – 0.97)	0.003	(0.83 – 1.53) [0.61, 1.81]
Arachidic	0.44 ± 0.0066 (0.42 – 0.48)	0.42 ± 0.0066 (0.39 – 0.45)	0.005	(0.35 – 0.48) [0.31, 0.52]
Eicosenoic	0.27 ± 0.0034 (0.26 – 0.29)	0.29 ± 0.0034 (0.29 ± 0.0034)	<0.001	(0.20 – 0.35) [0.16, 0.41]
Behenic	0.16 ± 0.010 (0.14 – 0.19)	0.14 ± 0.010 (0.13 – 0.17)	0.107	(0.071 – 0.27) [0.030, 0.28]

[1] More than half of the observations for lauric acid, myristic acid, myristoleic acid, pentadecanoic acid, pentadecanoic acid, palmitoleic acid, heptadecanoic acid, heptadecanoic acid, gamma linolenic acid, eicosadienoic acid, eicosatrienoic acid and arachidonic acid were below the assay limit of quantitation and were excluded from statistical analysis.
[2] Mean ± SE = least square mean ± standard error of the mean.
[3] The reference range is the range of values for the conventional corn varieties grown at the same 5 field sites. TI = tolerance interval specified to contain with 95% confidence, 99% of the population of conventional maize. Negative limits set to 0.

Table 8: Summary of vitamins and minerals in LY038 grain (combined sites)

Component[1]	LY038 mean ± SE[2] (Range)	LY038(-) mean ± SE (Range)	p-value	Reference Range 99% TI[3]
Folic acid	0.47 ± 0.029 (0.35 – 0.76)	0.40 ± 0.029 (0.33 – 0.54)	0.006	(0.24 – 0.60) [0.13, 0.59]
Niacin	19.49 ± 1.08 (17.40 – 21.81)	20.84 ± 1.08 (17.82 – 23.87)	0.177	(14.81 – 39.93) [5.17, 37.49]
Vitamin B1	4.07 ± 0.12 (3.52 – 4.64)	4.11 ± 0.12 (3.51 – 4.57)	0.677	(2.51 - 4.34) [1.80, 4.83]
Vitamin B2	1.50 ± 0.068 (1.10 – 1.74)	1.42 ± 0.068 (1.12 – 1.74)	0.116	(0.98 – 1.85) [0.77, 2.16]
Vitamin B6	5.93 ± 0.27 (4.63 – 6.95)	5.63 ± 0.27 (4.85 – 8.00)	0.22	(3.68 – 8.46) [2.50, 9.89]
Vitamin E	9.04 ± 0.87 (6.35 – 12.25)	10.63 ± 0.87 (8.30 – 13.35)	0.025	(6.94 ± 19.26) [0.26, 24.84]
Calcium % dwt	0.0046 ± 0.00022 (0.0039 – 0.0059)	0.0054 ± 0.00022 (0.0043 – 0.0064)	0.001	(0.0030 – 0.0075) [0.0013, 0.0076]
Copper	2.20 ± 0.12 (1.85 – 3.91)	1.78 ± 0.12 (1.53 – 2.03)	0.018	(1.12 – 2.58) [0.45, 2.97]
Iron	24.15 ± 0.74 (20.29 – 37.09)	23.40 ± 0.74 (20.13 – 29.75)	0.471	(15.39 – 27.88) [11.29 – 30.67]
Magnesium % dwt	0.14 ± 0.0036 (0.13 – 0.16)	0.14 ± 0.0036 (0.12 – 0.15)	0.214	(0.087 – 0.15) [0.075, 0.16]
Manganese	6.98 ± 0.52 (5.16 – 9.30)	7.72 ± 0.52 (5.70 – 9.64)	0.001	(3.33 – 10.22) [0.26, 12.49]
Phosphorus % dwt	0.37 ± 0.37 (0.31 – 0.44)	0.37 ± 0.37 (0.31 – 0.43)	0.966	(0.25 – 0.41) [0.21, 0.46]
Potassium % dwt	0.37 ± 0.011 (0.29 – 0.44)	0.38 ± 0.011 (0.34 – 0.45)	0.136	(0.32 – 0.46) [0.28, 0.46]
Zinc	26.19 ± 1.04 (22.01 – 31.22)	24.27 ± 1.04 (20.53 – 28.18)	0.002	(15.94 – 33.80) [8.94, 39.24]

[1] Shown as mg/kg dwt unless otherwise specified.
[2] Mean ± SE = least square mean ± standard error of the mean.
[3] The reference range is the range of values for the conventional corn varieties grown at the same 5 field sites. TI = tolerance interval specified to contain with 95% confidence, 99% of the population of conventional maize. Negative limits set to 0.

Table 9: Summary of secondary metabolites in LY038 grain (combined sites)

Component[1]	LY038 mean ± SE[2] (Range)	LY038(-) mean ± SE (Range)	p-value	Reference Range 99% TI[3]
Ferulic acid	2285.73 ± 86.33 (1988.8 – 2701.8)	2257.63 ± 92.44 (1970.88 – 2551.97)	0.827	(1935.8 – 3638.1) [1138.95, 3687.86]
Free lysine[4]	1351.13 ± 109.52 (921.9 – 1696.6)	25.99 ± 3.18 (18.39 – 40.21)	<0.001	(14.69 – 108.52) [0, 104.89]
Homoserine	11.18 ± 3.86 (5.48 – 29.32)	12.01 ± 5.55 (2.75 – 37.84)	0.906	(2.72 – 92.67) [0, 83.82]
L-Pipecolinic acid	28.72 ± 1.37 (22.72 – 35.35)	14.96 ± 1.58 (10.06 – 21.82)	<0.001	(2.71 – 42.14) [0, 45.15]
Saccharopine	650.29 ± 36.40 (499.30 – 818.42)	5.88 ± 0.90 (2.75 – 8.26)	<0.001	(2.71 – 42.14) [0, 23.00]
p-Coumaric acid	179.86 ± 22.83 (94.40 – 322.23)	150.70 ± 19.38 (76.22 – 217.80)	0.353	(141.55 – 433.26) [17.22, 472.67]
Phytic acid % dwt	0.68 ± 0.038 (0.36 – 0.90)	0.77 ± 0.038 (0.51 – 0.97)	0.099	(0.11 – 0.83) [0.12, 0.98]
Raffinose %dwt	0.13 ± 0.013 (0.078 – 0.18)	0.15 ± 0.013 (0.12 – 0.21)	0.089	(0.053 – 0.18) [0.0094, 0.22]

[1] Shown as ug/g dry weight unless otherwise specified
[2] Mean ± SE = least square mean ± standard error of the mean.
[3] The reference range is the range of values for the conventional corn varieties grown at the same 5 field sites. TI = tolerance interval specified to contain with 95% confidence, 99% of the population of conventional maize. Negative limits set to 0.
[4] Non-protein incorporated lysine.

Table 10: Summary of statistically significant differences outside of the reference tolerance interval (all sites)

Component	Site code[1]	LY038 mean (range)	LY038(-) mean	Mean difference (% of control)	p-value	Reference (99% tolerance interval)	Literature range / Historical range[2]
Free lysine µg/g dwt	1	1514.23 (1470.43 – 1584.33)	38.54	3828.81	<0.001	[0, 104.89]	-
	2	1317.71 (1277.99 – 1364.44)	23.76	5446.5	<0.001		
	3	994.42 (921.86 – 1042.10)	19.41	5023.87	<0.001		
	4	1349.11 (1200.09 – 1496.78)	23.5	5639.73	<0.001		
	5	1580.2 (1502.10 – 1696.61)	24.75	6284.14	<0.001		
	Combined	1351.13 (921.86 – 1696.61)	25.99	5098.13	<0.001		
Lysine % total amino acids	2	4.34 (4.04 – 4.50)	2.99	45.19	0.003	[1.85, 4.29]	2.0 – 3.8[3] / 2.08 – 4.18
	Combined	3.81 (3.08 – 4.50)	2.7	41.09	<0.001		
Copper mg/kg dwt	3	2.78 (1.88 – 3.91)	1.74	60.06	0.014	[0.45 , 2.97]	0.9 – 10[4] / 0.29 – 3.43
	Combined	2.2 (1.85 – 3.91)	1.78	23.11	0.018		
Total dietary fibre % dwt	2	31.41 (26.08 – 39.65)	18.4	70.71	0.007	[3.77, 39.08]	10.99 – 11.41[6] / 11.80 – 25.63
	Combined	20.77 (11.90 – 39.65)	15.99	29.87	0.042		

Component	Site code[1]	LY038 mean (range)	LY038(-) mean	Mean difference (% of control)	p-value	Reference (99% tolerance interval)	Literature range / Historical range[2]
Saccharopine µg/g dwt	1	590.95 (532.35 – 628.77)	6.91	8452.58	<0.001	[0, 23.00]	-
	2	678.53 (663.51 – 694.70)	7.37	9107.07	<0.001		
	3	583.16 (499.30 – 661.37)	2.76	21014.6	<0.001		
	4	625.55 (552.04 – 702.50)	5.94	10434.4	<0.001		
	5	773.28 (730.73 – 818.42)	6.4	11979	<0.001		
	Combined	650.29 (499 – 818.42)	5.88	10966.5	<0.001		
Total fat % dwt	Combined	3.86 (3.00 – 4.72)	4.42	-12.66	<0.001	[1.36, 4.67]	3.1 – 5.7[4]; 2.48 – 4.81[5] / 1.74 – 5.50
Folic acid mg/kg dwt	1	0.57 (0.53 – 0.62)	0.52	9.31	0.047	[0.13, 0.59]	0.3[4] / 0.28 – 0.86
	5	0.54 (0.43 – 0.76)	0.39	38.81	0.014		
	Combined	0.47 (0.35 – 0.76)	0.4	17.39	0.006		

[1] Site code (1) Jefferson, IA; (2) Benton, IA; (3) Clinton, IL; (4) Warren, IL; (5) York, NE; (Combined) all 5 sites combined.
[2] Historical range is from control samples (in some cases including commercial hybrid values) analysed in previous Monsanto Company Studies reported in (Sidhu *et al*., 2000; Ridley *et al*., 2002) and internal Monsanto reports
[3] (Watson, 1982)
[4] (Watson, 1987)
[5] (Sidhu *et al*., 2000)
[6] (Choi *et al*., 1999)

Table 11: Summary of α-aminoadipic acid levels in LY038 corn grain

Site	LY038 mean ug/g dwt (range)	LY038(-) mean ug/g dwt (range)	Reference substance mean ug/g dwt (range)
Jefferson, IA (1)	82.34 (78.58 – 89.32)	6.33 (6.19 – 6.46)	8.76 (5.59 – 13.45)
Benton, IA (2)	39.65 (36.59 – 42.41)	-*	-
Clinton, IL (3)	50.66 (46.56 – 54.68)	-	-
Warren, IL (4)	59.93 (44.62 – 67.74)	-	8.59 (7.83 – 9.36)
York, NE (5)	50.36 (48.27 – 51.79)	-	
Combined sites	56.59 (36.59 – 89.32)	6.33 (6.19 – 6.46)	8.73 (5.59 – 13.45)

* indicates values below the limit of quantitation (5 ppm)

Additional metabolites and catabolites of lysine

Five (homoserine, pipecolinic acid, free lysine, saccharopine and α-aminoadipic acid) of the seven selected additional compounds were consistently present in LY038 grain at levels above the limit of quantitation. Cadaverine and 2,6-diaminopimelic acid were below the limit of quantitation in all the grain samples analysed (LY038, LY038(-) and reference samples) so no values for these analytes are reported in the tables of compositional results.

At two of the five sites, levels of homoserine in LY038 grain were significantly different to the control grain, however this was not a consistent difference as at one of the sites homoserine levels in LY038 grain were significantly lower, and at the other significantly higher than the control grain. At all five sites, the homoserine levels were within the 99% T.I. of the reference grain.

LY038 grain contained significantly higher levels of pipecolinic acid than the control grain at all sites and when the sites were combined. However, the mean and range were within the 99% T.I. for conventional corn grain and therefore these differences were not considered to be biologically significant (Table 9).

As anticipated, free lysine levels were significantly increased, with levels in LY038 grain ranging from 921 – 1696 µg/g dry weight compared to an average of 25.99 µg/g dry weight in the control grain (Table 10). This represents a mean difference with the combined site data of greater than 5000% of the control. Lysine is an essential amino acid and is a common constituent amino acid in proteins. It is regarded as safe when added to animal diets at nutritional levels and may be safely used as a human food additive.

The level of saccharopine in LY038 grain was significantly higher than in the control and reference grain (Tables 9 and 10). The levels of α-aminoadipic acid in LY038 grain were also higher than those in the control, although no statistical analysis was performed on these results as the values for the control were generally below the limit of quantitation (Table 11). The significance of the results for these two compounds for the safety of food from LY038 grain is discussed below.

Saccharopine and α-aminoadipic acid

Substantial levels of α-aminoadipic acid have been reported in lentils (790 µg/100g fwt), lentil sprouts (19.15 mg/100 g fwt), garden peas (310 µg/100 fwt), garden pea sprouts (10.39 mg/100 g fwt), and lettuce (320 µg/100g fwt) (Nawaz and Sorensen, 1977; Rozan *et al*., 2001). Saccharopine has been found in asparagus and lettuce (400 µg/100g fwt), and edible mushrooms (102 µg/g) (Nawaz and Sorensen 1977; Oka *et al*., 1981).

In addition to a literature search, the Applicant confirmed the presence of α-aminoadipic acid and saccharopine in a variety of commonly consumed food products using the same validated methods as for the analytes in LY038 corn grain. The foods were purchased from local supermarkets in the St. Louis, Missouri metropolitan area over approximately one year. These analyses are shown in Table 12 and demonstrate a history of exposure to these lysine catabolites from the consumption of commonly available foods.

Table 12: α-aminoadipic acid and saccharopine levels in common foods

Lysine catabolite	Food	Number of samples analysed	Average level in crop µg/g (range)
α-aminoadipic acid	broccoli	2	490 (484 – 496)
	cauliflower	3	175 (17 – 315)
	green beans	1	141
	button mushrooms	3	637 (115 – 1074)
	LY038 corn	15	56.59 (36.59 – 89.32)
saccharopine	broccoli	2	122 (87 – 157)
	cauliflower	4	97 (87 – 157)
	button mushrooms	4	629 (385 – 986)
	LY038 corn	15	650 (499 –818)

The levels of α-aminoadipic acid found in LY038 corn grain ranged from 36.59 – 89.37 µg/g dwt with a mean of 56.59 µg/g. Compared to the levels found in other common plant foods, this level is not unusually high. The levels of saccharopine found in LY038 corn grain (499 – 818 µg/g dwt, mean 650 µg/g) are substantially higher than those found in broccoli or cauliflower, but similar to the level in button mushrooms.

Animals and humans are constantly exposed to these lysine metabolites as products of normal endogenous lysine metabolism and through the consumption of common foods.

There is no evidence to suggest that saccharopine or α-aminoadipic acid have any adverse effect in humans or animals when consumed as part of the diet. Both compounds are broken down and eventually become substrates for the tricarboxylic acid cycle, entering as acetoacetyl-CoA.

Further, the Applicant has performed a 90-day feeding study in rats with LY038 corn grain to demonstrate that the compositional changes in LY038 corn grain produce no long-term adverse effects. This feeding study is discussed in Section 6.

5.2 Conclusion

The grain of LY038 corn is considered to be compositionally equivalent to that of conventional corn with the exception of the intended increase in lysine and free lysine levels in the grain, and the associated increase in lysine-related catabolites, saccharopine and α-aminoadipic acid. No consistent differences in concentrations of essential amino acids other than lysine, including methionine, threonine and isoleucine (which share a portion of the lysine biosynthetic pathway in plants), were observed between LY038 grain and the control and reference grains.

6. NUTRITIONAL IMPACT

Study submitted
Taylor, M.L., Hyun, Y., Hartnell, G.F., Nemeth, M.A., Karunanandaa, K., George, B., Glenna, K.C. and Heydens, W.F. (2003) Sponser Summary of Report for Study 02-01-72-16 (Comparison of Broiler Performance When Fed Diets Containing LY038 x MON810, Negative Segregant Control, or Commercial Maize.). MSL-18883, an unpublished study conducted by Monsanto Company.

In assessing the safety and suitability of a GM food, a key factor is the need to establish that the food is nutritionally adequate and will support typical growth and wellbeing. In most cases, this can be achieved through an understanding of the genetic modification and its consequences, together with an extensive compositional analysis of the food.

To date, all approved GM plants with modified agronomic production traits have been shown to be compositionally equivalent to their conventional counterparts. Feeding studies with feeds derived from the approved GM plants have shown equivalent animal performance to that observed with the non-GM feed. Thus the evidence to date is that for GM varieties shown to be compositionally equivalent to conventional varieties, feeding studies with target livestock species will add little to a safety assessment and generally are not warranted.

However, for plants engineered with the intention of significantly changing their nutritional characteristics, such as LY038 corn, feeding trials with one or more target species may be useful to demonstrate wholesomeness for the animal. In this case, the compositional analyses found corn line LY038 to be equivalent to conventional corn with the exception of changes related to the increased lysine trait, so the Applicant conducted two feeding studies with this corn, one using chickens (the target species) and a second using rats.

6.1 Feeding study in chickens (42-days)

Traditional corn-soybean meal based chicken feeds are typically deficient in lysine and require the addition of supplemental lysine for optimal animal growth and production.

The supplemental lysine is commonly provided from commercially available lysine sources in the form of lysine monohydrochloride or lysine sulphate. Both these lysine sources have been demonstrated to be highly bioavailable. LY038 corn was developed to provide an alternative source of additional lysine in chicken diet formulations, thereby reducing or eliminating the need to include this supplement.

Study aim

To assess the nutritional value of LY038 corn when used as an animal feed, a growth study was conducted in fast growing broiler chickens, the main target species of LY038 corn feed. The fast growing broiler is a useful species for feeding studies because relatively small changes in growth rate, feed efficiency, and/or carcass measurements as a result of a change in nutritional or health status can be readily detected (Hammond *et al*., 1996; Taylor *et al*., 2003). The study was designed to determine the growth response in chickens fed a diet containing LY038 corn compared to diets containing conventional corn varieties and lysine-supplemented diets containing conventional corn varieties.

Study conduct

Cobb x Cobb 500 male broilers were used in a 42-day study to compare the feeding value of LY038 grain (0.40% lysine) to negative segregant control LY038(-) grain (0.27% lysine), and four lots of conventional corn grain (0.24-0.25% lysine). Test, control and reference grains were analysed for nutrient composition as a basis for diet formulation and to assess suitability for use in the feed trial. This included analyses of mycotoxins and pesticides, as well as event-specific PCR to confirm the presence or absence of the *cordapA* gene. A starter diet containing 64% (w/w) corn was fed from days 0-21 and a grower/finisher diet containing 70% corn was fed from days 21-42. The different diets are shown in Table 13.

Diets containing LY038 corn and lysine supplemented diets contained 1.06% and 0.90% total lysine in the starter and grower/finisher diets respectively. These lysine levels were below the National Research Council (National Research Council, 1994) recommendations and the Illinois ideal lysine to amino acid ratio and were selected so that birds would be growth responsive to changes in dietary lysine quantity and availability. The non-supplemented diets containing control grain, LY038(-), or conventional corn grain contained 0.95% and 0.80% total lysine for the starter and grower-finisher diets respectively. The other five amino acids essential for broiler growth (methionine, cystine, arginine, tryptophan and threonine) were present at levels designed to meet or exceed 105% of the Illinois ideal lysine to amino acid ratio based on 1.06% and 0.90% lysine levels for the starter and grower-finisher diets respectively, assuring that lysine would be the first limiting amino acid in these diets.

Ten pens of 10 birds were fed each treatment diet. Broilers were weighed by pen on days 0, 21 and 42, and individually at study termination (day 43, 44 or 45). Feed intake per pen was determined for the same intervals as bird weight gain, allowing calculation of feed efficiency by pen, based on total weight of surviving broilers in the pen or adjusted to include weight gain of any broilers that died or were culled during the study. At study termination, all surviving birds were processed to determine carcass yield and meat composition. Fat pad measurements were taken for each bird. One broiler per pen was randomly selected for breast and thigh meat quality assays.

Results

Performance parameters

Performance parameter means and standard error of the mean for all treatment diets are shown in Table 13.

In general, performance parameters were similar ($p > 0.05$) across the broilers fed diets containing LY038 corn and those fed lysine supplemented diets containing LY038(-) or conventional corn varieties. Average body weight gains for days 0 – 21, days 21 – 42 and days 0 – 42 were comparable among treatments. No significant differences were observed when comparing LY038 corn to the lysine-supplemented diets in regard to body weight gain by bird or by pen. No differences in feed intake and feed efficiency between the LY038 corn treatment and the lysine-supplemented treatments were noted for the entire study period (0-42 days), except for one of the five conventional diets (Burrus 789). No observed unintended effects occurred when feeding LY038 corn to broilers and the bioavailability of the lysine in LY038 diets was similar to that in diets supplemented with crystalline lysine.

Bio-efficacy of LY038 diets was demonstrated by performance differences ($p < 0.05$) across broilers fed diets containing LY038 corn versus diets not supplemented with lysine containing LY038(-) corn or other conventional corn varieties. Significant differences in performance between broilers fed the LY038 diet and all other non-supplemented diets were noted for the entire test period for weight gain (kg/bird or kg/pen), feed intake (kg/bird or kg/pen), feed efficiency (kg/kg) and adjusted feed efficiency (kg/kg).

Carcass measurements

Carcass parameters for the entire 42-day study are summarised in Table 14. The weight of broiler carcass components were increased ($p < 0.05$) in birds fed lysine supplemented LY038(-) and conventional corn diets compared to the equivalent non-supplemented diets, demonstrating the responsiveness of the broiler chicken to additional lysine. The measured carcass components, expressed as a percentage of live bird weight basis (% fat pad, % wings, % drums, % thighs and % breast), were also increased with lysine addition to the lysine-deficient control and reference corn diets. A consistent increase in breast meat protein on a weight or percentage basis, further illustrates the improved carcass quality observed with lysine supplementation of lysine deficient broiler diets.

Bio-efficacy of the lysine in LY038 corn was demonstrated by improved yield of total chilled carcass and prime parts, including breast, drums, thigh, and wings for birds fed LY038 corn diets compared to birds that received control or reference corn non-supplemented diets. The LY038 corn containing diets had similar ($p > 0.05$) weight, percentage and chemical composition of measure carcass components from birds fed LY038 corn versus those of birds fed lysine supplemented conventional corn diets.

Table 13: Treatment diets and broiler performance

Treatment			Mortality[b] + removed	Avg. Wt. gain per bird (kg)	Adjusted Gain:Feed[c]
Grain	Dietary lysine[a]	Lysine addition		Mean (SEM)	
Burrus 789 (reference)	0.94 / 0.80	No	1	1.554 (0.037)	0.448 (0.020)
Pioneer 34M94 (reference)	0.95 / 0.80	No	1	1.808 (0.056)	0.498 (0.008)
SC1122 (reference)	0.95 / 0.80	No	0	1.666 (0.036)	0.467 (0.011)
SC1091 (reference)	0.95 / 0.80	No	0	1.655 (0.035)	0.480 (0.015)
LY038(-) (control)	0.98 / 0.80	No	1	1.544 (0.054)	0.453 (0.015)
Burrus 789 (reference)	1.06 / 0.90	Yes	1	2.175 (0.049)	0.580 (0.005)
Pioneer 34M94 (reference)	1.06 / 0.90	Yes	1	2.186 (0.042)	0.564 (0.006)
SC1122 (reference)	1.06 / 0.90	Yes	0	2.241 (0.041)	0.570 (0.008)
SC1091 (reference)	1.06 / 0.90	Yes	0	2.195 (0.047)	0.564 (0.004)
LY038(-) (control)	1.06 / 0.90	Yes	1	2.120 (0.043)	0.544 (0.011)
LY038 (test)	1.06 / 0.90	No	1	2.193 (0.029)	0.545 (0.010)

[a] Calculated lysine level in Starter / Grower-Finisher diet
[b] Number of deaths + culls from day 7-42
[c] Adjusted gain:feed = (pen weight gain + weight of dead or removed birds) / pen feed intake

Table 14: Summary of broiler carcass data at study termination

Treatment		Live wt[a] (kg)	Fat pad wt (kg)	Chill wt (kg)	Wings wt (kg)	Drums wt (kg)	Thighs wt (kg)	Breast wt (kg)
Grain	Lysine addition	Mean (Standard Error of the Mean)						
Burrus 789	No	1.666 (0.036)	0.023 (0.001)	1.112 (0.030)	0.143 (0.003)	0.180 (0.004)	0.213 (0.007)	0.204 (0.008)
Pioneer 34M94	No	1.888 (0.056)	0.028 (0.002)	1.295 (0.041)	0.161 (0.004)	0.208 (0.006)	0.248 (0.008	0.252 (0.010)
SC1122	No	1.764 (0.038)	0.027 (0.001)	1.195 (0.031)	0.151 (0.003)	0.190 (0.005)	0.226 (0.006)	0.231 (0.007)
SC1091	No	1.750 (0.044)	0.023 (0.001)	1.178 (0.035)	0.150 (0.004)	0.191 (0.006)	0.225 (0.007)	0.224 (0.008)
LY038(-)	No	1.628 (0.054)	0.020 (0.001)	1.082 (0.041)	0.140 (0.004)	0.178 (0.006)	0.206 (0.009)	0.197 (0.010)
Burrus 789	Yes	0.2250 (0.066)	0.037 (0.001)	1.595 (0.052)	0.190 (0.004)	0.241 (0.007)	0.297 (0.013)	0.361 (0.014)
Pioneer 34M94	Yes	2.270 (0.032)	0.035 0.002)	1.608 (0.025)	0.190 0.001)	0.243 (0.003)	0.300 (0.005)	0.364 (0.009)
SC1122	Yes	2.314 (0.048)	0.037 (0.002)	1.630 (0.041)	0.196 (0.004)	0.247 (0.005)	0.300 (0.008)	0.367 (0.013)
SC1091	Yes	2.270 (0.044)	0.034 (0.001)	1.609 (0.033)	0.190 (0.003)	0.242 (0.004)	0.296 (0.007)	0.369 (0.010)
LY038(-)	Yes	2.193 (0.039)	0.031 (0.001)	1.538 (0.031)	0.186 (0.003)	0.239 (0.004)	0.287 (0.005)	0.330 (0.009)
LY038	No	2.267 (0.031)	0.031 (0.001)	1.588 (0.023)	0.190 (0.002)	0.245 (0.004)	0.296 (0.007)	0.349 (0.005)

[a] Live weight is after approximately 12 hours feed withdrawal

Conclusion

No unexpected effects on bird performance or health were observed in the birds fed LY038 corn grain. The LY038 corn diet was comparable to conventional corn diets supplemented with lysine in terms of performance and carcass measurements, demonstrating the bioavailability and efficacy of the increased lysine in LY038 corn. The LY038 corn diet gave superior results compared to non-supplemented conventional corn grain.

6.2 Feeding study in rats (90-days)

The Applicant also conducted a 90-day feeding study in rats to demonstrate that the compositional changes in LY038 corn grain had no long-term effects on the growth and wellbeing of animals consuming this grain. FSANZ has reviewed this study, however the study has been granted confidential status under the Freedom of Information Act and detailed results cannot be released.

In summary, lysine maize LY038 grain was fed to groups of 20 male and female rats in a 90-day feeding study at dietary levels up to 33% of weight (w/w). No mortality occurred during the study. There were no test article-related adverse effects observed based on daily clinical observations, weekly body weight, and food consumption. At the end of the study, all animals were killed and subjected to further analysis. No test article-related adverse effects were seen in terminal clinical pathology tests, organ weight measurement, or gross and microscopic pathology.

The study authors concluded that administration of LY038 corn grain to rats for 90-days at 33% (w/w) in the diet had no effects on the growth or health of the animals.

Acknowledgements

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ATTACHMENT 3

Summary of Public Submissions

First Round

200 submissions were received. The option supported by each submitter is indicated in brackets following their name.

1. Australian Food and Grocery Council (Tony Downer) (2)

- Believes FSANZ should not have accepted this application as it is primarily intended for animal feed, however, support option 2.
- Recommends that the safety assessment takes into consideration the potentially very low dietary exposure to this product.
- Believes that due to the very low levels of this corn expected in the food supply, it will be very difficult for manufacturers to comply with labelling regulations and therefore should be exempt.

2. Food Technology Association of Victoria (David Gill) (2)

3. New Zealand Food Safety Authority (Carol Inkster) (-)

- Agrees that the issues raised in the IAR need to be considered.
- Asks that all supporting studies be summarised and referenced in the Draft Safety Assessment Report.

4. Queensland Health, Environmental Health Unit (G Bielby) (-)

- Believes that the approval of this application will impact on monitoring resources in the jurisdiction, and that as costs associated with testing are significant, this should not be left solely to the jurisdictions, but should be a national activity.
- Suggests that a national repository be set up for testing methodology and standards.
- Believes a multiscreen methodology needs to be developed for GMOs.

5. PSRG – Physicians and Scientists for Responsible Genetics (Jean Anderson) (1)

- Opposes GM food, believe the molecular characterisation is incomplete, concern about the lack of independent long term feeding studies, concern about the novel proteins, corn products are widely consumed on a daily basis and could potentially affect all consumers.

6. Victorian Department of Human Services (Victor Di Paola) (-)

7. NSW Food Authority (Michael Apollonov) (2)

- Supports the issues raised by Queensland Health

8. South Australian Department of Health (Kirsten Potoczky) (-)

- Suggests that a variation to standard 1.5.2 is the only means to approve this product for use in food, rather than this being the most cost-effective means as stated in the Initial Assessment.
- Notes that as LY038 is not to be grown in Australia approval will have no effect on growers.
- Notes that the issue of disruption to trade rely on LY038 being found to be safe, which it had not been at Initial Assessment. These issues have been addressed in the Draft Assessment Report.

9. GE Free New Zealand in Food and Environment (Claire Bleakley) (1)

- Opposes GM food. Concerned that they have not seen the supporting studies.
- Supports the submission by NZIGE

10. GE Free New Zealand in Food and Environment, Nelson Branch (Susie Lees) (1)

- Opposes GM Food
- Supports the submission by NZIGE
- Concerned about pesticides, antibiotics, potential toxicity or allergenicity, CaMV promoter and believe that the current labelling system is inadequate

11-14. GE Free Northland in Food and Environment – (Linda Bench), T C Vallings, Baerbel Leeker and Irmgard Habl (1)

- Oppose GM food
- Support the submissions by NZIGE and PSRG

15-27. South Coast Environment Society Inc (Robert Guyton), J Carapiet, Quentin Jamieson, Sue Kedgley, Jack Mooney, Jane Pearce, Virginia Richardson, Anne Smith, Annie Stuart, Katherine Vasbenter, Raymond Vogt, Carol Walker, Sky Williams (1)

- Support the submission of the NZIGE

28-29. Waikato GE free network (Carla Davis and Catherine Iremonger) (1)

- Concern about potential allergenicity,
- inadequate pretesting, lack of human clinical trials, lack of long term human feeding studies, ineffective separation systems.
- Would like an independent assessment to be done on the application.
- Feel that approval of this corn will undermine the right of consumers to choose.

30. Green Society Inc (Hans Grueber) (1)

- Would like studies to prove it will be safe for 100 years
- Concern for the economic future of NZ as it may lose its green reputation

31. Me Aroha Waiheke Foundation (Consumer group) (C Lehwenz) (1)

- Opposes GM food

32. Soil and Health, Canterbury Branch (Annmarie Banchy) (1)

- Opposes this Application
- Inadequate pre-testing, absence of long term studies in humans, ineffective separation systems. Would like an independent review before approval of LY038 is considered

33. Tainui Hapu ki Whaingaroa Environmental Committee (Angeline Greensill) (1)

- Believes that GM food is tampering with nature
- Corn has been a stable part of the diet for many generations
- Is concerned there may be health or environmental effects

34. Organic Farm New Zealand (OFNZ) Wainue Pod members (Betty Kettle) (1)

- Supports NZIGE submission.
- Opposes the application

35. New Zealand Institute of Gene Ecology (Jack Heinemann) (-)

- Submitted an analysis of the data supplied to FSANZ by Monsanto to support A549
- Supports further analysis of LY038 before approval
- Raised questions about the appropriateness of FSANZ assessing animal feed
- Recommendations of the NZIGE are addressed in Attachment 4

36. Jews for GE free food (Hilary Phillips) (1)

- Concerned that GM foods may not be kosher

37. Russell Poulter (University of Otago) (-)

- Supports a draft assessment being done.

38. Murray Lane (2)

- Supports GM crops and food when they have been shown to be safe
- Agrees with the recommendation of the Royal Commission on Gene Technology to proceed with caution

39. Tom Atkinson (2)

- Supports approval of LY038

40. Jeremy Ayres (-)

- Notes that FSANZ is considering A549

41. Kent Briggs (1)

- Not opposed to GM technology, but believes long term safety studies are needed

42–184. Doreen Adams, Shushila Ajani, Erwin Alber, Chris and Maria Aulman, Dee Austring, Annemarie Banchy, Amy Bankoff, Rosemary Bartle, Ruth Begg, Linda Bench, Taleb Bench-Kanjou, Graham Bennett, Dave Beere, Jocelyn Bieleski, Paul Bradley, Lisa Bridson, Paul Brimecombe, Kalani Bruce, Tony Bruce, Berthine Bruinsma, Carolyn Campbell, Lars Chresta, Mairead Ni Chonaola, Trish Coates, Marion and Peter Corby, Dayahn Cornelius, Mona-Lynn Courteau, Hugh Cronwright, Victoria Davis, Colin Day, Charles Drace, Emily Dennis-Bishop, Amy Donovan, K Du Pont, Helen Eggers, Jodene Fabian, Rhonda Fearnley, Sue Ferrabee, Lillian Fougere, Shari French, Ann Fullerton, Bernard Gadd, Noeline Gannaway, Jan Gerritsen, David Graham, Zelka Grammer, William Green, David Grove, Rosemary Grueber, Malibu Hamilton, Elizabeth Harrington, Annette Hart, Colin Hewens, David Hodges, David Holmes, Maureen Howard, Stella Hughes, Rita Hunt, Shane Hyde, Loraine Johnstone, Ange Jones, Hilary Jones, Kylie Jones, Oraina Jones, Rosie Kaplan, Andy Kirk, Tracy Kuck, Paula Lambert, Jane Landman, Anne Larsen, Marlene Laureys, J. Ruth Lawson, Susie Lees, Raylene Lodge, G. Mabbs, Jane Mabey, Leslie Macdonald, Rachel Mackeson, Dugald MacTavish, Lisa Marshall, Vicki Martin, Wendy McGuinness, Mike McCree, Emily McDowell, Shona McKee, Rose MacKinnon, Corinne and Donald McBride, Mary McCammon, Carol McLean, Mandy McMullin, Mario McMillan, Johanna Metz, Shane Metzler, Robert Mignault, Valerie Morse, Lora Mountjoy, Patsy O'Brien, Wim Oosterhoff, Nicki Owers, Pam Parsons, Don Paterson, Fiona Paton and Sam Storey, Lea Sturmer, Jennifer Pearson, Neville Pearson, Liz Peters, Richard and Tracey Pettinger, Trish Puharich, Stephen Richardson, Joan Roesch, Ian Roger, Tara Ross-Watt, David Rouse, Mr Royal, James Russell, Brian Scrafton, Amanda Semb, Andrew Sharpe, Mark Sidebotham, Simon, Neil Sloan, Anita Smith, Gillian Somerville, Hanne Sorensen, Campbell Sturrock, Julia Struyck, Ali Symmons, Sarah Therkleson, Myles Thomas, Colin Thomson, Phyllis Tichinin, Max Tobin, Mike Trott, Kevin Tutt, Ed Tye, Clare Tyler, Grant Walters, Liz Westbrooke, Betty Wheeler, Melanie White, Phil Wilkie, Steve Williams, Jeanette Wilson (1)

Generally opposed to GM foods. The issues raised include:

- Concern about inadequate pre-testing and absence of long-term human feeding studies (over more than one generation)
- Concern that there is no proof of the benefits to human and animal health, as well as concerns about health and safety. Request of 'hard evidence' that there will be no unexpected impacts on human, animal and plant lives.
- Concern with segregation of GM crops.
- Concern that approval will take away freedom of choice to eat GM-free food.
- Concern about the stability of the introduced genes
- Advocate the use to the precautionary principle.
- Concern about effects of consumption of animals that have been fed LY038 corn.
- Support the submission by the New Zealand Institute of Gene Ecology
- Concern that the supporting studies have been conducted by the applicant and are not independent peer reviewed studies.
- Request clearer traceability systems to track GM foods in the food supply.

- Concern that GM foods could damage New Zealand's GM free image.
- Worried that Monsanto has withheld unfavourable test data in the past.
- Believe that current labelling requirements for GM foods are not strong enough.
- Concern that this application was not widely advertised and therefore many people are unaware of it.
- Concern about the use of chemicals in the food supply and the effect this has on the environment
- Suggest that official labels be introduced through legislation for GE Free and Organic Foods.
- Suggest a subsidy for healthy foods.
- Worried about damage to the organic food industry
- Concern about potential environmental damage and effects on biodiversity
- Concern for members of the public with heightened susceptibility to allergens and processed foods
- Concern regarding lack of independent testing of GM crops
- Concern that GM foods may get into foods as diverse as margarine, cereal, cooking oil, sweeteners and alcoholic beverages.
- Fear that GE modified products will create a generation of infertile people in 4-5 generations from now
- Concern about multinational companies, such as Monsanto.
- Believe that submissions to New Zealand Food Standards are a waste of time as FSANZ is dominated by Australia
- Worried that GM food may change the nature of human intestinal flora
- Worried about increasing antibiotic resistance among bacterial populations.
- Believe that New Zealand should impose an indefinite moratorium on GM until all safety and liability issues are resolved.

185. Anthony Peacocke

- Believes that an animal feed should not be approved for human consumption

186. Pauline Bailey

- Concern that people may be allergic to LY038
- Requests that FSANZ ensure this corn is safe as a routine part of the food supply and not just as an occasional inadvertent ingredient.
- Opposed to application

187-194. Sheena Beaton, Morag Brownlie, C. Cooper, Davian Horlor, Daniel Meares, Alex Taylor, Nerine Walbran, Patricia Waugh

- Inadequate safety data
- Ineffective separation and labelling systems deny consumers choice
- Opposed to application

195. Peter Hunt

- Opposed to application for a number of reasons – insufficient safety data, corn is a staple food and this will affect many people, potential biosecurity threat, wants the right to purchase organic food, recommends that LY038 be subjected to the same testing requirements as medicines.

196. Shaun Lee

- concerned about liability issues

197. Graham Smith

- Opposed to application
- Concerned that once approved as an animal feed it can enter food supply
- Concern about potential for new allergies

198. JR Collins

- Believes there is currently inadequate pretesting and that the impact analysis is incomplete

199. Lee Short

- Believes it is unacceptable for animal feed to contaminate human food.
- Believes there is currently inadequate pretesting and that the impact analysis is incomplete
- Opposes the application

200. Peter Thompson

- Believes the application is nothing more than an attempt by Monsanto to protect itself if LY038 is found to enter the food supply.
- Believes that there is no demonstrable social benefit and therefore the application should be rejected

Second Round

Fourteen submissions were received. Issues that have not been addressed previously (e.g. FAQs on the FSANZ website) have been addressed in the Final Assessment Report.

Submitter	Option	Comments
South Australian Department of Health (Kirsten Potoczky)	2	Notes that most imported corn products would be refined foods. Notes that as LY038 is a high-value animal feed, it is likely to only be present in human food inadvertently at low levels. Lysine in LY038 does not pose a risk to public health Comments on the decreased levels of vitamin E and linoleic acid, although these are within the range found in conventional corn. Suggests that further analysis (e.g. histopathology) of the broilers in the feeding study could have lent weight to the safety assessment. Notes that the rat feeding study was confidential.
Friends of the Earth (New Zealand)	1	Believe that that approval of A549 is a foregone conclusion, however note their opposition to GM foods and crops. Concerned that the use of LY038 corn may not be restricted to animal feed once food approval is given. Note that no other country has approved this GM crop. Concerned about the effects of the transgene on soil ecology. FSANZ notes that this is beyond our scope and would need to be considered by the Office of the Gene Technology Regulator or the Environmental Risk Management Authority if LY038 corn were to be grown in Australia or New Zealand. Currently LY038 is not permitted to be grown in either country. Concerned that the studies supporting the application were conducted by the applicant Believe that animal feeding studies are necessary as are human trials with LY038
Paul Elwell-Sutton	1	Concern that the novel DNA may enter the tissues of animals that consume GM feeds. Concerned that food products from animals fed GM corn would not have to be labelled as genetically modified Believes currently labelling requirements for GM foods are not sufficiently stringent
Australian Food and Grocery Council	2	The AFGC support the approval of LY038 and the current labelling requirements for GM foods.
Queensland Health (Gary Bielby)	2	Comments that costs of monitoring and enforcing GM food legislation continue to be a problem to jurisdictions Suggests a national enforcement strategy for GM foods, including education, should be considered

Submitter	Option	Comments
Department of Human Services, Victoria (Victor Di Paola)	2	No comment
NSW Food Authority (Jenine Ryle)	2	No particular concerns with this application
GE Free New Zealand (Claire Bleakley)	1	Concern about potential introduction of LY038 into the human food supply Concern about the recent allergenicity study in GM peas (CSIRO, 2005) Concerns over the entire safety assessment process and whether FSANZ should a have a role in assessing animal feed.
New Zealand Food Safety Authority (NZFSA)	2	No comment
Food Technology Association of Victoria	2	No comment
Renessen LLC (Monsanto) (Kevin Glenn)	2	Provided letters from a variety of US academics and industry groups in support of the approval of LY038
Ivan Jeray	1	Concern that GM foods have not been shown to be safe Cites the example of the CSIRO field pea Concern over the adequacy of the labelling requirements for GM foods
Kathryn Liddell	1	Concerned that there is insufficient evidence of the safety of GM foods Concerned that FSANZ is accepting the US approval rather than conducting our own assessment
Centre for Integrated Research in Biosafety (Associate Professor Jack Heinemann)	1	Made a significant submission including 94 recommendations, which are discussed in Attachment 4.

ATTACHMENT 4

FSANZ response to the submission on the Draft Assessment Report from the Centre for Integrated Research in Biosafety

FSANZ received a detailed submission on the Draft Assessment Report for A549 from the Centre for Integrated Research in Biosafety (INBI, previously the New Zealand Institute of Gene Ecology, NZIGE). The submission, which includes 94 recommendations in relation to the safety assessment of food from high lysine corn, follows comments previously submitted by the NZIGE on the Initial Assessment Report. These comments were addressed by FSANZ at Draft Assessment.

The current submission from INBI asserts the following:

1. the scientific studies on LY038 do not prove it to be as safe as conventional corn;
2. LY038 has a substantially different potential to create food hazards during cooking;
3. hybrids with LY038 could create significant additional food hazards;
4. the novel protein has no history of safe use;
5. LY038 has been tested as an animal feed, not a human food;
6. FSANZ has accepted a standard of evidence of safety that is below what it could request under international guidelines; and
7. a recommendation to amend the Code does not follow from a case-by-case assessment.

After consideration of the evidence, INBI expresses the view that:

- too much legitimate scientific uncertainty exists;
- there is considerable evidence of probable harm in comparison to conventional corn;
- the recommendation is inconsistent with Codex;
- more studies should be requested from the Applicant;
- any approval for high lysine corn should be restricted to food derived directly from the specific line evaluated (LY038) and not include food from hybrid lines; and
- FSANZ should impose an actively managed post-market monitoring program.

FSANZ Response

General comments

High lysine corn has been developed primarily for animal feed, where it will be used to replace conventional corn-soy based swine and chicken diets which are characteristically deficient in lysine and require the addition of supplemental lysine for optimal animal growth and performance. Identity preservation methods will be used to segregate this product from conventional grain, however it is possible that a small percentage of LY038 grain may be inadvertently co-mingled with corn destined for the human food supply.

As a consequence, and following consultation with FSANZ, Monsanto Australia Limited is seeking approval for food derived from corn line LY038 in the Code. FSANZ has therefore conducted a pre-market safety assessment on high lysine corn according to the assessment guidelines applied to all other GM foods.

FSANZ's safety assessment of GM food is part of an overall risk analysis designed to identify whether a hazard, nutritional or other health and safety concern, is present in a GM food (hazard identification), and if present, to examine information on its nature and severity (hazard characterisation). The hallmarks of this approach are: case-by-case assessment; consideration of both intended and unintended effects; and comparisons with conventional foods having an acceptable standard of safety.

To standardise this approach and ensure consistency, FSANZ has developed Guidelines for the Safety Assessment of Genetically Modified Foods which describe the general approach and framework for a GM food safety assessment. FSANZ also has regard to the Codex Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants, which is broadly consistent with the FSANZ guidelines. The Codex guideline was developed to facilitate a consistent and harmonised scientific approach to GM food safety assessment.

Case-by-case assessments are necessary because the key issues requiring consideration in a safety assessment will often depend on the nature of the genetic modification and the type of food. For this reason, the application of the safety assessment guidelines should remain flexible in order to address the specific and unique issues that can arise as a result of different genetic modifications. This does not mean that less rigorous assessments may be undertaken, but rather recognises that certain types of information may be unnecessary in some cases or that different types of information may sometimes be required.

High lysine corn has been assessed according to FSANZ's guidelines as well as the Codex guideline and in the same rigorous manner as all previous GM food safety assessments. The conclusion from this assessment is that food derived from corn line LY038 is as safe and wholesome as food derived from other corn varieties. Contrary to the INBI assertion, the increased levels of lysine in the corn grain are not a safety concern.

FSANZ has undertaken a comprehensive analysis of all the issues raised in the INBI submission (see response to specific issues below) and found no scientific justification for the expressed safety concerns. FSANZ is satisfied that the level of evidence provided by the Applicant is sufficient to demonstrate the safety of the food, and on this basis there is no reason to consider imposing special conditions on any approval for food derived from high lysine corn.

Comments on the INBI submission

In dealing with the INBI critique, FSANZ has observed and noted a number of inconsistencies in the discussion and inaccuracies in reporting the scientific literature.

For example, while FSANZ has been criticised by INBI for deviating from the Codex guideline, INBI have repeatedly suggested the use of experimental techniques that are not endorsed by Codex or other intergovernmental organisations, and which have not been validated for the purpose of safety assessment (e.g. RNA microarray). While advocating the use of methods which are still requiring development and yet to be validated, INBI criticises well-established methodologies such as bioinformatics which are endorsed by Codex and the FAO/WHO as part of an overall strategy for assessing potential allergenicity.

FSANZ has also noted that the INBI submission contains a number of factual errors. On more than one occasion, a journal article has been cited by INBI as evidence supporting a particular view, however when FSANZ has cross-checked the statements in the INBI submission with the cited article, the results and conclusions drawn by the author of the journal article are contrary to those represented in the INBI submission. Such misinterpretations of the literature and speculative discussion have been used to give the erroneous impression of a heightened degree of uncertainty around the safety of food from LY038.

For example, INBI has raised the issue of the potential for possible novel Maillard reaction products to be allergenic. The INBI submission notes (page 45) there is evidence that some allergens are attenuated or removed by heat or during processing, while other allergens (such as AraH2, one of the dominant peanut allergens) become more potent on heating (Gruber *et al.*, 2005). The INBI submission cites Gruber *et al.* (2005) and asserts that 'In this example, even the minor allergen Ara H1/2 (peanut agglutinin) was converted into an IgE-binding product after incubation with sugar at elevated temperatures'. This is an incorrect interpretation of the results reported in this study. Gruber *et al.* (2005) found that the majority of peanut allergic patients tested showed an IgE specific response to untreated peanut agglutinin. Heating peanut agglutinin in the presence of sugar either had no effect, or, in one case, gave a reduced IgE response. The authors conclude that the 'allergenic activity of peanut agglutinin might be decreased by Maillard-type reactions' (Gruber *et al*., 2005).

In another example, the INBI submission cites Panigrahi *et al.* (1996) as evidence of lysine formed anti-nutrients in maize as a result of stackburn (page 49). Panigrahi *et al.* (1996) report that maize discoloured by stackburn resulted in reduced weight gain and lower efficiency of feed utilization in broiler chicks. The results reported by Panigrahi *et al.* (1996) have been incorrectly interpreted by INBI. Stackburn deterioration of maize quality during storage resulted in a 52% reduction in lysine. As lysine levels are already limiting in maize, reductions in lysine bioavailability through the Maillard reaction reduces the metabolisable energy, leading to deterioration in growth performance. This reduction in availability of an essential amino acid due to stackburn is not evidence of formation of anti-nutrients but rather a reduction in available nutrients. Panigrahi *et al.* (1996) conclude that 'it is, therefore, probable that reductions in both the ME (metabolisable energy) value and lysine and arginine contents account for most of the deterioration in growth performance observed in the broiler chick trial'. The conclusion in the INBI submission that 'lysine in corn cannot be generally regarded as safe (GRAS)' is a misrepresentation of the Panagrahi *et al.* (1996) study. As noted by INBI earlier (p44), 'glycation of lysine and protein reduces the nutritional value of the food'.

The INBI submission is also selective in its use of information. Recently, Monsanto researchers published three papers on a proteome analysis of *Arabidopsis thaliana* (Ruebelt *et al*., 2006a, 2006b and 2006c). The first paper reported the analytical methodology, the second an assessment of natural variability in the proteome of different non-GM *Arabidopsis* varieties and the third paper was an assessment of alterations in the proteome of GM *Arabidopsis* plants. When the papers are read together it is clear the analyses indicated that any variations in the proteome of the GM plants were within the natural range of variation found in the non-GM plants. INBI referred only to the first and third papers, and cited these as evidence that Monsanto has the ability to conduct proteome analysis on GM plants. However, INBI did not report the fact that the study authors conclude that the analysis provided no results that would be meaningful or useful to inform a safety assessment.

References:
Gruber P, Becker WM and Hofmann, T (2005). Influence of the Maillard Reaction on the Allergenicity of rAra h2, a Recombinant major Allergen from peanut *Arachis hypogaea*, Its Major Epitopes, and Peanut Agglutinin. *J. Agric Food Chem*. **53**:2289-2296

Panigrahi S, Bestwick LA, Davis RH and Wood CD (1996). The nutritive value of stackburned yellow maize for livestock: tests in vitro and in broiler chicks. *Br. J. Nut.* **76**:97-108.

Ruebelt, M. C., Leimgruber, N. K., Lipp, M., Reynolds, T. L., Nemeth, M. A., Astwood, J. D., Engel, K. H. and Jany, K. D. (2006a). Application of Two-Dimensional Gel Electrophoresis To Interrogate Alterations in the Proteome of Genetically Modified Crops. 1. Assessing Analytical Validation. J. *Agric. Food Chem.* 54:2154-2161.

Ruebelt, M. C., Lipp, M., Reynolds, T. L., Astwood, J. D., Engel, K. H. and Jany, K. D. (2006b). (2006b). Application of Two-Dimensional Gel Electrophoresis To Interrogate Alterations in the Proteome of Genetically Modified Crops. 2. Assessing Natural Variation. *J. Agric. Food Chem.* 54:2162-2168

Ruebelt, M. C., Lipp, M., Reynolds, T. L., Schmuke, J. J., Astwood, J. D., DellaPenna, D., Engel, K. H. and Jany, K. D. (2006c). Application of Two-Dimensional Gel Electrophoresis To Interrogate Alterations in the Proteome of Gentically Modified Crops. 3. Assessing Unintended Effects. *J. Agric. Food Chem*. 54:2169-2177.

Response to the recommendations 1-94 from INBI

R1: The Authority should report the DNA sequence of the Glb1 promoter in event LY038. Since the Applicant claims that it is the endogenous corn promoter, the actual sequence should not be a commercial secret.

The Applicant sought confidentiality for the DNA sequence of the insert in LY038 and flanking regions. Although individual genetic components of the construct used for transformation of LY038 may be publicly available, the combination of elements is unique. The information provided to FSANZ therefore comprises the results of extensive research and intellectual property required for both the commercial viability and regulatory authorisation of corn line LY038. The request for confidentiality was approved because it fulfils the criteria for confidential commercial information set out in the FSANZ Act.

R2: The Authority should report the true breeding history for both LY038 and LY038(-) that includes the precise point at which the two lines segregate. From this history, the Authority should evaluate whether there is certain evidence that LY038 is more closely related to LY038(-) than H99.

The breeding history of LY038 and LY038(-) has been clarified in Section 3.1 of the Safety Assessment (Attachment 2 to the Final Assessment Report). The breeding tree diagram presented in this section clearly shows that LY038 and LY038(-) have the same parental plant and are therefore more closely related to each other than to the more distant parental line H99 (from which R0 plants in the breeding tree diagram were derived).

R3: The Authority is requested to have the anomalous result in figure 6 of MSL-19871 explained, or have the analysis redone, before accepting this as evidence of either a single insertion in LY038 or the absence of insertions in LY038(-).

Figure 6 in Study MSL-19871 shows a Southern blot of genomic DNA purified from LY038, LY038(-), and 5 different corn varieties used in producing LY038, probed with DNA specific to the *cordapA* coding region.

The slight variation observed in the intensity of one band representing conventional corn line 'Inbred A' could be due to a number of experimental variables including inconsistent loading of DNA, and does not change the overall results, which are consistent with the conclusion that there is one DNA insert in LY038.

The safety of food derived from LY038 was determined by evaluation of the totality of scientific evidence from multiple strands of data, and was not based on one Southern blot.

R4: Consistent with CAC/GL 45-2003, 'the sensitivity of all analytical methods should be documented'. Therefore, the Authority should report the minimum size of target DNA that all probes could detect at a minimum stringency of 0.5 copies per genome.

The Codex guideline (CAC/GL 45-2003) stipulates that the sensitivity of all analytical methods should be documented. However, Southern blots provide a qualitative rather than a quantitative analysis and therefore the guideline does not apply.

R5: We recommend that that Authority require a range of analytical methods that includes a combination of FISH, fiber-FISH and Southern analysis.

Currently, fluorescence in situ hybridisation (FISH) techniques are primarily used in studies on animal cells to provide information on genome organisation. These techniques are highly specialised and are certainly not well-established for use with plant cells and results in these circumstances can be variable and unreliable. A recent study in maize using a FISH technique found that the shortest probe that could be detected was 3.1 kb and that sequences closer than ~100 kb could not be resolved (Wang, Harper and Cande, 2006). Therefore, at this stage, analyses such as FISH would not add substantially to the information obtained from more established methods such as Southern blot analyses using multiple probes.

Reference:

Wang CJ, Harper L and Cande WZ (2006) High resolution single-copy fluorescence in situ hybridisation and its use in the construction of a cytogenetic map of maize chromosome 9. *Plant Cell* **18(3):**529-44.

R6: The issue of background hybridisation could be fully proved by sequencing the light bands visible in the Southern blots. The Authority should therefore base their final conclusion on the results of sequencing.

This recommendation refers to Southern blots of restriction digested-genomic DNA from LY038, LY038(-) and 5 conventional varieties of corn which contribute to background genetic information on LY038.

The corn genome is large and restriction digests of genomic DNA consist of a multitude of DNA fragments of variable size. When subjected to agarose gel electrophoresis, the digested DNA appears as a smear rather than discrete bands. As the genomic DNA in any hybridising band on the Southern blot would include multiple co-migrating genomic fragments of similar sizes, sequencing a particular band would not be a reasonable or effective method for characterising LY038. It is also relevant to note that the probes used in Study MSL19871 also hybridise with endogenous corn sequences.

FSANZ considers that more useful information is gained from a comparison of the pattern of bands for LY038, the comparator and the conventional controls, recognising that a background of non-specific hybridisation would be expected using genomic DNA digests. Due to the technical difficulties in separating multiple co-migrating bands, and the availability of other supporting molecular characterisation data, FSANZ does not consider sequencing of numerous genomic fragments would add significantly to the safety assessment and therefore is not warranted.

R7: The Authority should clarify whether additional inserts are present in LY038 by requiring additional studies on the high molecular weight fragments in MSL-19871.

Plant genomic DNA is notoriously difficult to purify and is often bound to carbohydrates and cellular remnants carried over from extraction of the plant cells. These contaminants can affect the digestibility of genomic DNA with restriction endonucleases. The high molecular weight regions on some Southern blots often represent non-specifically degraded DNA or only partially digested DNA.

These technical details however do not detract from the evidence provided by a number of Southern blots using a variety of probes, which consistently indicated the presence of one DNA insert in corn line LY038.

R8: The Authority should explain how it has the confidence that the experimental procedures used by the Applicant would have detected an insert the size of the *loxP* site in an unknown location at 0.5 copies per genome.

FSANZ considers that in the absence of detectable unintended changes to the phenotype of LY038, the presence of an insert the size of a 34 base pair (bp) *loxP* site at 0.5 copies per genome is highly unlikely to affect the safety of food derived from high lysine corn. It is important to acknowledge that plant genomes of conventional non-GM crops such as corn are peppered with mobile genetic elements and could never be expected to remain static through multiple generations of breeding.

R9: The Authority should verify that the residual *loxP* site in LY038 is not processed by the *cre* recombinase.

The *loxP* site consists of 34 bp made up of two 13-bp inverted repeats and an asymmetrical 8-bp spacer. The *cre* recombinase can catalyse recombination between two *loxP* sites with identical 8 bp spacers. There is the possibility that recombination might occur between the residual *loxP* site in LY038 and another identical site in the corn genome (should such a site exist), if *cre* recombinase is present. However, the *cre* recombinase is not present in LY038. Moreover, the potential for recombination between *loxP* sites decreases as the physical distance between the sites increases; sites on different chromosomes recombine much less efficiently than linked sites.

Gross chromosomal changes due to such a recombination event would most likely result in unviable gametes, and significant changes to phenotype might be expected in any viable offspring.

Although this type of gross chromosomal rearrangement can occur experimentally, there would be no reason for a developer to intentionally combine a line such as LY038 with a *cre* line to produce a commercial crop that might be vulnerable to this problem. FSANZ considers this to be a remote possibility.

R10: The Authority should provide evidence that all novel RNA species have been identified, characterised and tested for food safety.

R11: We recommend that the Authority require a complete microarray description of the LY038 transcriptome, compared to the unmodified control, for proper hazard identification.

R12: The Authority should require the Applicant to report on the results of microarray analyses using the mouse genome and RNA extracts from the intestinal cells of mice fed LY038.

Microarray technology is a powerful tool to study gene expression and the potential value of such technology for the safety assessment of GM foods is currently being investigated by a number of groups. Preliminary results from these studies suggest that this method may be used effectively to screen for altered gene expression, and, at the same time, may provide information on the nature of the detected alterations. However, at this point in time, a number of limitations exist: microarray standards need to be established; databases need to be established to generate information regarding the extent of natural variability for each data point; and new software needs to be developed to handle the very large data sets that are generated. So, while microarray techniques may prove useful to identify differences among tissues between a food component from a GM product and its conventional counterpart, the relevance to the safety assessment still remains to be established. Therefore, currently, such methods are not yet suitable for use in safety assessment.

The use of such techniques was considered by a FAO/WHO expert consultation on the Safety Aspects of Genetically Modified Foods of Plant Origin (WHO 2000), where it was recognised that such techniques may contribute to the detection of differences in a more extensive way than targeted chemical analysis. However, it was also recognised that such techniques are not yet fully developed and validated and have certain limitations. For this reason, the Codex guideline does not refer to the use of such techniques.

More recently, this issue has been examined in the context of undertaking nutritional and safety assessments of foods and feeds nutritionally improved through biotechnology (ILSI 2004). In relation to microarray technology, it was concluded that its usefulness for the identification of unintended effects in GM crops depends largely on documented information about natural variations in gene expression levels in crop plants, which is still lacking.

FSANZ considers techniques such as microarray technology to still be experimental and as such it would not be appropriate to require such studies in support of the safety of a food.

References:

WHO (2000). *Safety aspects of genetically modified foods of plant origin*. Report of a Joint FAO/WHO Expert Consultation on Foods Derived from Biotechnology, Geneva, Switzerland, World Health Organization.

ILSI (2004). Nutritional and safety assessments of foods and feeds nutritionally improved through biotechnology. *Comprehensive Reviews in Food Science and Food Safety* **3:** 38-104.

R13: While the Applicant continues to rely upon unvalidated methods (e.g. bioinformatics as described above) for hazard identification, the Authority should make the insertion and flanking sequences publicly available for evaluation by those who may then bring more relevant analyses to bear.

The DNA sequence data have been accepted by FSANZ as valid confidential commercial information (see response to R1 above) and therefore are not publicly available.

FSANZ considers that the INBI submission is placing too much weight on the overall importance of bioinformatics in the allergenicity assessment. At present, there is no definitive test that can be relied upon to predict allergenic response in humans to a novel protein. Because of this, it is recommended by Codex that an integrated, stepwise, case-by-case approach be used. This approach, as elucidated in the Codex guideline, takes into account the evidence derived from several types of information and data, since no single criterion is sufficiently predictive. The determination of the extent to which a novel protein is similar in structure to a known allergen, using bioinformatic analysis, is just one part of this assessment. The assessment also includes consideration of the source of the novel protein, pepsin resistance, specific serum screening (if the protein originates from a source known to be allergenic, or has sequence homology to a known allergen), exposure to the novel protein, and the effects of relevant food processing. The results from these studies are then used to reach a conclusion as to the likelihood of the novel protein being a food allergen.

R14: The Authority should report not just total lysine content of foods, but free lysine content of foods and provide comparisons with conventional corn, especially H99. The Authority should also consider the ratio of carbohydrate to free lysine.

R15: The Authority should provide the people of Australia and New Zealand with reliable data demonstrating that processing and cooking temperatures normal to products that could contain this corn are as safe as products derived from conventional corn, particularly the parental varieties of LY038.

R16: The Authority should request an analysis of all novel AGE content or AGE concentrations, including Maillard reaction products and glycotoxins, that could arise from cooking, storage or processing of LY038 corn compared to parental varieties.

The Maillard reaction (also known as the browning reaction, glycation and non-enzymatic glycosylation) is a broad term that encompasses a wide range of reactions between sugars (carbonyl groups) and amino acids (free amino groups). These complex reactions produce hundreds of products, including those responsible for the cooked colour and flavour of many foods, such as bread crusts, chocolate, roasted meats and fried foods (McGee, 2004). Advanced Glycation End-products (AGEs) and Maillard reaction products (MRPs) are produced during cooking, particularly frying and baking at high temperatures with low moisture. The particular range of MRPs produced will be influenced by the particular composition of proteins, sugars and fat of the food and also by the cooking method and duration of cooking. The Maillard reaction also occurs *in vivo* and during prolonged storage of food.

There is conflicting evidence for the health benefits or harm due to dietary MRPs, reflecting the wide range of Maillard products that exist. For example, Kitts and Hu (2005) suggest the antioxidant activity of MRPs can have a protective effect on cells, as well as enhancing food shelf life.

The MRPs in bread crusts, notably pronyl-L-lysine and N-epsilon carboxymethyllysine, have also been shown to enhance antioxidant capacity and lead to an increase in chemopreventive enzymes (Somoza *et al*., 2005). In contrast, recent studies have found that the presence of acrylamide, a known carcinogen, in some fried foods is due to the reaction of the amino acid asparagine with sugars (e.g. Becalski *et al*., 2004). There is still no clear link between dietary acrylamide exposure and cancer incidence, despite the long history of consumption of browned foods (Blank, 2005). The possible health effects of acrylamide in food are areas of ongoing research.

The INBI submission raises concerns about possible health risks of novel MRPs that may be produced on processing of LY038, particularly because of the high levels of free lysine, and because the epsilon-amino group on lysine makes it a preferred substrate for Maillard reactions.

There is no reason to believe that free lysine would undergo more extensive Maillard type reactions than protein-incorporated lysine. Therefore, total lysine is a more appropriate way to report lysine levels, rather than separating free lysine from protein-incorporated lysine.

The INBI submission asserts that 'LY038 cannot be compared to non-corn foods because non-corn foods with higher lysine levels have much lower levels of carbohydrates'. It is unrealistic to expect that a crop with an intentionally altered nutritional profile can be compared to a conventional food with an identical nutritional profile. While the production of MRPs during cooking depends on the content of both protein/amino acids and carbohydrates (such as the Becalski *et al.* 2004 study cited by INBI), there are also anomalies to this generalisation, such as meat, which produces relatively high levels of MRPs despite having a high protein level but low carbohydrate level (Goldberg *et al.*, 2004). There is not a simple correlation between the ratio of protein to carbohydrate and AGE content of food or the increase in AGE content post-cooking (Koschinsky *et al.*, 1997). Factors such as cooking method, duration and moisture levels have a significant influence on MRP formation.

The identification and characterisation of Maillard reaction products (MRPs) in food is a growing field of research. While the most common products of Maillard chemistry have been identified, the complete profile of MRPs of *any* food, conventional or otherwise, has not been determined and is limited by available technology (Gerrard, 2006). Even if such an analysis were technically achievable, it is unlikely to contribute substantially to a safety assessment. 'Many foods contain substances that would likely be found harmful if subjected to conventional approaches to safety testing' (CAC/GL 45-2003). In addition, the MRP profile produced by cooking corn will vary depending on the other ingredients in the processing milieu and the processing method, as is true for any other food ingredient.

For these reasons, FSANZ does not consider it necessary that a new suite of studies be performed with cooked LY038 corn as the results of these would be unlikely to add further to the safety assessment.

Furthermore, the increased levels of lysine in LY038, and their potential to form AGEs, should be considered in the context of the total diet. Although the levels of lysine in LY038 are significantly increased (almost doubled) compared to conventional corn, corn is a poor source of lysine.

Even if all corn products consumed by Australian and New Zealander consumers were derived from LY038 corn, this would represent an insignificant increase in lysine consumption as Australian and New Zealand populations consume only relatively small quantities of corn-derived products.

Data from the 1995 Australian National Nutrition Survey (NNS) indicates that maize was consumed in the form of maize flour by 2394 consumers (17% of the 13858 survey respondents). Consumption for Australian maize consumers aged 2 years and above was 20 grams per day at the mean and the 95th percentile consumption for consumers was 67 grams per day[6]. For New Zealand, maize was consumed by 1066 consumers (23% of the 4636 survey respondents). Consumption for New Zealand maize consumers aged 15 years and above was 14 grams per day at the mean and the 95th percentile consumption for consumers was 60 grams per day.

Mean intake of maize (approximately 20 grams per day in Australia) is a better representation of intake over a longer period of time than the 95th percentile consumption. If the entire intake of maize came from LY038 corn grain, lysine intakes would increase by 50 mg/day for consumers of maize. When compared with lysine intake from other sources, (e.g. 700-2800 mg in 100 g of cheese, or 250 mg in 100 g broccoli) this increase would have no impact on the overall diet. See the FSANZ response to R.62 for lysine levels in other food types.

References:

Becalski A, Lau BPY, Lewis D, Seaman SW, Hayward S, Sahagian M, Ramesh M, and Leclerc Y (2004) Acrylamide in French Fries: Influence of Free Amino Acids and Sugars. *J. Agric. Food Chem.* **52**:3801-3806

Blank, I. (2005) Current Status of Acrylamide Research in Food: Measurement, Safety Assessment, and Formation. *Ann. N.Y. Acad. Sci*. **1043**: 30-40.

Gerrard, JA (2006) The Maillard reaction in food: process made, challenges ahead – Conference report from the Eight International Symposium on the Maillard Reaction. *Trends in Food Sci. Tech.* **17**:1287-1291

Goldberg T, Cai W, Peppa M, Dardaine V, Baliga BS, Uribarri J and Vlassara H (2004) Advanced glycoxidation end products in commonly consumed foods. *J. Am. Diet. Assoc* **104**:1287-1291.

Kitts, D.D. and Hu, C. (2005) Biological and chemical assessment of antioxidant activity of sugar-lysine model maillard reaction products. *Ann. N.Y. Acad. Sci*. **1043**: 501-512.

Koschinsky, T., He, C-J., Mitsuhashi, T., Bucala, R., Liu, C., Buenting, C., Heitmann, K. and Vlassara, H. (1997) Orally absorbed reactive glycation products (glycotoxins): an environmental risk factor in diabetic nephropathy. *Proc. Nat. Acad. Sci. USA*. **94**: 6474-6479.

McGee, H. (2004) McGee on Food and Cooking: An encyclopedia of kitchen science, history and culture. Hodder & Stoughton, London.

Somoza, V., Wenzel, E., Lindenmeier, M., Grothe, D., Erbersdobler, HF and Hofmann, T. (2005) Influence of feeding malt, bread crust, and a pronylated protein on the activity of chemopreventive enzymes and antioxidative defense parameters in vivo. *J. Agric. Food Chem*. **53**: 8176-8182.

[6] These figures were derived using the FSANZ dietary modelling computer program, DIAMOND. The consumption figures include maize from all sources in the diet including mixed foods. Some of the foods consumed included cornflakes, taco shells, tortillas, corn chips, cornflour and anywhere cornflour is used (biscuits, custards, sauces) and maize based pasta.

R17: The Authority should justify its conclusion that lysine levels in a genetically modified variety of corn can be considered safe by comparison to lysine levels in unrelated food sources, such as red meat, chicken, eggs, cheese, broccoli, lentils and fish.

FSANZ uses the comparative approach to assess the safety of a new GM food. A key step in this process is the comparison of a new GM food to its conventional counterpart; however this is not a safety assessment in itself. It simply provides a starting point for the identification of any differences that may raise safety and/or nutritional concerns. Any identified differences are then subject to further assessment. Since the lysine levels of LY038 are intentionally higher than those of conventional corn varieties, it is appropriate to consider the possible impact of the increased lysine levels by comparison to other conventional foods with similar levels of lysine.

This approach, whereby high lysine corn is compared to unrelated food sources, is entirely consistent with the Codex guideline (CAC/GL 45-2003). The guideline states that when a modification results in a food product 'with a composition that is significantly different from its conventional counterpart, it may be appropriate to use additional conventional foods or food components (i.e. foods or food components whose nutritional composition is closer to that of the food derived from recombinant-DNA plant) as appropriate comparators to assess the nutritional impact of the food'.

R18: The Authority should require that the Applicant supplement application A549 with a complete set of long-term, chronic, sub-chronic and acute toxicity feeding studies and allergenicity studies using cooked products derived from LY038, and compared to the parental varieties.

FSANZ has considered the issue of cooked versus uncooked products (see response to R14-16) and concluded that studies with cooked products are not necessary, nor would they provide meaningful results.

In relation to the issue of animal testing, it is well accepted that it is not feasible to apply traditional toxicological testing procedures to whole foods, since they cannot be fed to animals at the levels required for toxicological testing due to their bulk. Animals fed a single whole food for extended periods of time may suffer nutritional imbalances that can confound the interpretation of the study results. The difficulties in applying traditional toxicological testing to whole foods are, in part, the rationale for using the comparative approach in risk assessment, which focuses consideration on differences between the new food and its conventional counterpart.

R19: The Applicant should conduct dietary AGE mouse feeding studies equivalent to those reported by Peppa *et al.* (Peppa et al., 2003b).

The Peppa *et al*. (2003) study utilised the NOD (Non-Obese Diabetic) mouse model for human type 1 diabetes in feeding studies comparing a low Advanced Glycation Endproduct (AGE) diet to a high AGE diet. A commercial mouse chow was cooked to elevate the AGE content five-fold. The study found that rats on the high AGE diet had an earlier onset of diabetes and a higher mortality than the low AGE control group.

The relevance of this study to the safety assessment of LY038 is tenuous at best. The five-fold increase in AGE content used in the mouse study is extreme, and LY038 corn, cooked as part of a normal diet, would not make a substantial change to dietary AGE intake.

Reference:

Peppa M, He C, Hattori M, McEvoy R, Zheng F and Vlassara H (2003) Fetal or Neonatal Low-Glycotoxin Environment Prevents Autoimmune Diabetes in NOD Mice. *Diabetes* **52**:1441-1448.

R20: The Authority should justify its claim with reference to recommendations of international food safety agencies that for LY038, with its significantly different nutritional profile, additional feeding studies are not required.

FSANZ does not consider that further feeding studies are justified. While it is reasonable to assume that processed corn products containing LY038 may contain an altered profile of AGE/MRPs compared to conventional corn, this is unlikely to have a significant impact on the overall diet of consumers.

Any health risks of dietary MRPs will primarily be influenced by the overall diet (i.e. the range of foods consumed, of which corn is a relatively minor component as discussed in the response to R.14-R.16) and food preparation methods (i.e. blanching and steaming versus frying and baking). That is, if current recommendations for a healthy diet are followed, any influence of AGEs from high-lysine corn, either positive or negative, would be expected to be minimal.

FSANZ is also mindful of the Codex Principles for the Risk Analysis of Foods Derived from Modern Biotechnology (CAC/GL 44-2003) which state that 'another consideration in deciding the need for animal studies is whether it is appropriate to subject experimental animals to such a study if it is unlikely to give rise to meaningful information'.

R21: The Authority should explain why it has accepted comparisons between LY038 and another product of gene technology with no history of safe use, LY038(-), rather than the CAC recommended standard of a comparison to conventional parental varieties.

R22: The Authority should explain why LY038(-) was used as a control instead of the more closely related conventional variety, and parent, H99.

FSANZ has examined the breeding tree of LY038 and considers that LY038(-) is an appropriate control corn line to use for the molecular characterisation and compositional analysis, and does not agree that H99 is more closely related to LY038.

No single maize parental inbred line could serve as a near isogenic line for LY038. A number of inbred corn lines contributed to the genetic background of LY038 as it was necessary to cross the transformant with a second maize line in order to increase the seed return, and to cross with the *cre* containing line to remove the *nptII* gene. The Codex guideline suggests that the appropriate comparator should be determined on a case-by-case basis.

The molecular analyses provide sufficient evidence to indicate that LY038(-) does not contain the novel gene construct. Due to the number of conventional breeding steps between H99 and LY038 and LY038(-), FSANZ does not consider H99 to be an appropriate comparator.

R23: If the Authority accepts LY038(-) as a control, then it should explain how it verified the absence of small inserts in the LY038(-) with experiments that would detect the 34 bp loxP sequence at 0.5 copies per genome.

The evidence suggests that LY038(-) does not contain any novel DNA, however it is unlikely that a 34 bp *loxP* site present at 0.5 copies per genome would be detected. However, if such a site were present, FSANZ does not hold the view that this would invalidate the use of LY038(-) as a comparator because it has been established through extensive molecular analyses that LY038(-) is negative for the novel traits being evaluated, and thus can be regarded as equivalent to a conventional corn, irrespective of whether or not a remnant *loxP* site remains.

R24: The Authority should provide a statistical analysis of the reference ranges per site.

The purpose of the reference range is to allow additional comparison between the composition of a genetically modified variety and conventional varieties of the same commodity.

The reference range is used when statistical differences are found between a GM variety and the appropriate non-GM control variety. By comparing the composition of a GM variety with a reference range, the biological significance of any statistical differences can be assessed.

A statistical analysis of the composition of each of the reference corn varieties at each site would add nothing to the safety assessment.

R25: The Authority should base its recommendation to amend the Food Code based on a proper comparison between LY038 and its parental varieties, H99, Inbred A, B and C grown under identical conditions in at least five test sites repeated in at least two growing seasons.

FSANZ, and other food regulatory agencies, have determined LY038(-) to be an appropriate comparator for LY038. Although the varieties H99, Inbred A, B and C have all contributed to the genetic background of both LY038 and LY038(-), the genetic background of LY038(-) is closer to that of LY038 than is any one of the above mentioned conventional varieties of corn. The closer the comparator is in genetic background to LY038, the more sensitive the comparison will be in detecting unintended effects directly related to the introduced novel traits.

FSANZ has considered data from five sites across three corn growing regions in the USA, with each site containing three replicates. This is considered sufficient information to support the compositional analysis of LY038 in this case.

R26: If the Authority is satisfied with the existing compositional data, we then ask it to indicate how it determine the values provided by the Applicant were as scientifically sound as those used in international guidelines.

The literature ranges used by the OECD, which represent information from a variety of sources from different years, are very useful where no other relevant data exist.

However, in some cases, published literature ranges do not exist (e.g. for total dietary fibre in the case of maize proximate analysis, only a single value was available to the OECD at the time the consensus document was written).

In the case of LY038, the reference range supplied by Monsanto was based on corn varieties grown in the same year at the same locations as LY038 and LY038(-). These data are more relevant to LY038 than the general ranges supplied in the OECD consensus document, and for this reason FSANZ has accepted the use of these reference ranges in preference to the literature ranges on the OECD Consensus Document on maize. If specific data did not exist or were unavailable it would be appropriate to use the OECD ranges as a basis for comparison.

R27: The Authority should evaluate the use of other novel foods as comparators in safety assessments and determine how long a novel food must be used safely before it is considered having a history of safe use.

There is no internationally agreed definition of what period of time would constitute a history of safe use.

The varieties of corn used to establish the reference range for Application A549 are conventional corn varieties in commercial production and as such are regarded by FSANZ to be a suitable benchmark by which to measure the relative safety of LY038.

INBI has noted that some of these varieties may only have been available for a few years. It is important to mention that commercial varieties of corn change regularly as conventional breeding is used to produce hybrids and particular varieties with desirable traits. These varieties have been bred from existing corn varieties and are as safe as any other corn.

INBI also noted that Health Canada has determined a number of plants derived through conventional breeding to be 'novel'. The Health Canada definition of novel food is not one that is used by Australia and New Zealand.

R28: The Authority should require the proximate analysis of maize starch, grits and flour derived from LY038.

Proximate analysis has been performed on LY038 corn kernels. FSANZ considers that this is sufficient information as a variety of different food products are produced from the kernels, including starch, grits and flour. The constituents of the kernels are expected to be representative of the constituents of food derived from them.

Milling (in the production of flour) would not alter the composition, nor would the composition be expected to change in grits, which are also produced from the kernel. A proximate analysis of starch would add nothing to the safety assessment as starch is composed of amylose and amylopectin and would contain little, if any, other components.

R29: The Authority should justify its conclusion that lysine catabolite levels in a genetically modified variety of corn can be considered safe by comparison to lysine levels in unrelated foods.

See response to R17. The Codex guideline (CAC/GL 45-2003) suggests that where a genetic modification results in a food product with composition significantly different from its conventional counterpart, it may be appropriate to use additional conventional foods or food components as appropriate comparators to assess the nutritional impact of the food.

In the case of LY038, a comparison to other types of food (including broccoli and button mushroom) was considered appropriate to give an indication of the levels of lysine catabolites in other food types. No further conclusions were drawn from this comparison.

R30: The Authority should provide quantitative evidence of cadaverine levels in LY038, perhaps by requiring NMR combined with chemometrics and univariant statistics to achieve more sensitive detection. If it does not, then the Authority should require feeding studies using LY038 cooked and processed as normal for human food to assess the potential for cadaverine at elevated levels in corn to produce food hazards.

Cadaverine is a biogenic amine which can be produced through the breakdown of lysine. It is found in fresh and fermented fish products and inhibits diamine oxidase. As diamine oxidase is involved in the degradation of histamine, cadaverine is thought to potentiate the toxic effects of histamine, present in inappropriately stored fish products, resulting in histamine poisoning. The level at which histamine causes histamine poisoning is not clear. Nor is the level of cadaverine capable of potentiating histamine toxicity in food known at this stage (Taylor, 1986).

Cadaverine levels in different foods vary significantly and may change over the life of the food product. A small survey conducted by the Department of Human Services, Victoria, showed that two samples of freshly purchased fish contained 8 and 21 ppm cadaverine (Kerr *et al*., 2002). A larger survey of fermented fish and fish products (e.g. pickled fish, fish sauce and fish paste) was also conducted. Of the 37 samples tested, cadaverine values ranged from approximately 10 ppm to over 7,000 ppm (den Brinker, Rayner and Kerr, 1996).

As cadaverine is a breakdown product of lysine, LY038 corn was analysed for this compound. Both LY038 and LY038(-) corn lines were below the limit of quantitation (LOQ) of 5 ppm (5 mg/kg) for cadaverine. This is below levels found in fresh fish and is not expected to have any impact on the safety of LY038 corn for human consumption.

Reference:

den Brinker C, Rayner C and Kerr M (1996) Investigation of biogenic amines in fermented fish and fish products. Public Health Division, Victorian Government Department of Human Services

Kerr M, Lawicki P, Aguirre S and Rayner C (2002) Effect of Storage Conditions on Histamine Formation in Fresh and Canned Tuna. Public Health Division, Victorian Government Department of Human Services

Taylor S (1986) Histamine food poisoning: toxicity and clinical aspects. *CRC Critical Reviews in Toxicology* **91**:128.

R31: The Authority should assess the sensitivity of those on monoamine oxidase inhibitors to measured levels of cadaverine in a diet composed of LY038 corn.

As described at R30, cadaverine can inhibit diamine oxidase. It is also true that at high doses some monoamine oxidase inhibitors (MAOIs) can have an inhibitory effect on this enzyme, an effect thought to be responsible for some of the side effects of MAOI antidepressants (IPCS, 2000).

However as cadaverine is found in a variety of foods and has not been found at quantifiable levels (LOQ 5 ppm) in LY038 corn, there is no reason to suppose that any clinically significant effect would be observed from the consumption of LY038 corn.

Reference:
IPCS (2000) Monoamine oxidase inhibitors http://www.inchem.org/documents/pims/pharm/pimg025.htm (Accessed 10 July 2006).

R.32: The Authority should report total pipecolic acid levels in LY038 and not just L-pipecolic acid levels.

R.33: The Authority should assess the contribution the intestinal flora will make to pipecolic acid levels in consumers who eat corn with high levels of lysine, free lysine and pipecolic acid.

R.34: The Authority should explain how it has considered the impact of pipecolic acid in high lysine corn on those suffering from chronic hepatic encephalopathy.

Pipecolic acid levels in LY038, although higher than LY038(-), are within the reference range of other corn varieties (see table below). These levels are therefore not considered biologically relevant or of concern to public health and safety.

Lysine from any source in the diet may be broken down to pipecolic acid by bacteria in the gut. These bacteria can produce both L- and D-forms of pipecolic, which can also be converted from one form to the other.

Chronic hepatic encephalopathy (CHE) is a complex neuropsychiatric condition. While high pipecolic acid levels may be present in chronic liver disease, there is no evidence that CHE is caused by dietary pipecolic acid. Part of the treatment for CHE may involve a protein-free or low-protein diet.

Zellweger syndrome, also mentioned in the INBI submission, is a rare congenital peroxisomal disease. There is no cure for this disease and it usually results in death in affected infants. These infants are seriously ill and may have altered levels of many metabolites due to their inability to carry out a number of cellular functions usually performed by peroxisomes. There is no suggestion that dietary pipecolic acid (either L- or D-forms) causes this disease.

Extract from Table 9 in A549 Safety Assessment Report

Component (µg/g dry weight)	LY038 mean ± SE[1] (Range)	LY038(-) mean ± SE (Range)	p-value	Reference Range 99% TI[2]
L-Pipecolinic acid	28.72 ± 1.37 (22.72 – 35.35)	14.96 ± 1.58 (10.06 – 21.82)	<0.001	(2.71 – 42.14) [0, 45.15]

[1] Mean ± SE = least square mean ± standard error of the mean.
[2] The reference range is the range of values for the conventional corn varieties grown at the same 5 field sites. TI = tolerance interval specified to contain with 95% confidence, 99% of the population of conventional maize. Negative limits set to 0.

R35: The Applicant has reported absolute amounts (by weight) of the amino acids in its most recent study (MSL-18881) but the Authority has accepted the statistical analysis based on %AA. The Authority should present the statistical analysis based on absolute amounts by weight.

FSANZ accepts the analyses provided as adequate to assess the composition of LY038. A simple transformation of absolute amounts to % values does not influence the results of the statistical analysis.

R36: The Authority should provide evidence that hybrids with the LY038 event have the same absolute amounts of glutamate, free lysine, saccharopine and α-aminoadipic acid as LY038 to assure the Authority that LY038 has no physiological behaviours that are unique to its genetic background with regard to lysine catabolism in seed.

R37: The Authority should address the difference in expected ranges of total and free lysine (as reported in A549) and the higher values already known to exist in hybrids created by the Applicant by explaining how it has determined what absolute levels of these compounds in corn could be a cause for concern.

Food from a hybrid plant line does not warrant a separate pre-market safety assessment if food from the parental GM plant lines have already been subject to a safety assessment. FSANZ considers the food safety risks posed by the conventional breeding of GM plants are no different from those arising from the conventional breeding of non-GM plants. It is widely recognised that unintended changes may occur during conventional breeding, however the products of conventional breeding have a long history of safe use and are not regulated by FSANZ.

R38: The Authority should provide evidence that LY038 and any hybrids with the LY038 event have the same absolute amounts of SAM and spermidine, and report on feeding studies using LY038 corn prepared as per normal for human consumption to assure the Authority that LY038 has no physiological behaviours that are unique to its genetic background with regard to lysine catabolism in seed.

The INBI submission states that elevated levels of cadaverine in corn might have physiological effects including potentially leading to S-adenosyl-L-methionine (SAM) deficiency and the suppression of spermidine and spermine synthases.

However, as the compositional analysis has shown that levels of cadaverine in both LY038 and LY038(-) are below the level of quantitation, there is no scientific basis for speculating that levels of SAM and spermidine might be altered.

FSANZ does not consider separate studies on cooked/processed LY038 products to be necessary for the safety assessment (see responses to R14 and R18).

R39: The Authority should report on the characterization of the 35 kDa bands found in preparations of cDHDPS produced *in-planta*.

R40: The 34 and 35 kDa forms should be demonstrated to be free of all post translational modifications, not just the addition of sugars.

R41: The 34 and 35 kDa forms should be used in allergenicity and toxicity studies.

The 35 and 34 kDa 'bands' are not distinct from the 33 kDa band and do not represent different proteins. The amount of protein loaded and the resolution of the gel mean that a single protein may appear across a small range (33-35 kDa) on the gel. FSANZ does not require further analysis.

R42: The Authority should be able to confirm the existence of molecular data to demonstrate that the modification made to the amino acid sequence, in this case amino acid 266, does not affect its post-translational modification or range of biochemical functions.

The amino acid sequences of the *E. coli* produced and plant produced cDHDPS are identical at position 269 (position 266 in the amino acid sequence of cDHDPS in SwissProt). Both proteins have a leucine residue at this position. There was an error in the amino acid sequence reported in both MSL-18365 and MSL-18565. The Applicant has amended these reports to reflect the error. The correct sequence was used for the bioinformatic analyses. No post-translational glycosylation was observed for either bacterial or LY038 produced cDHDPS.

R43: We recommend that the Authority require a complete proteomic analysis of LY038 grain using 2D gel electrophoresis and MS and an account of all changes between LY038 and its non-modified parent. The Applicant has demonstrated in a recent series of publications that it has the technology to do such profiling (e.g. Monsanto studies Ruebelt et al., 2006a, Ruebelt et al., 2006b). Each change should be identified as either a variant of cDHDPS or an unintended change in the modified plant. All variant forms of cDHDPS should be characterized for glycosylation or other posttranslational modifications (5.3.17).

FSANZ considers analyses such as proteome analysis to still be experimental and as such it would not be appropriate to request such studies in support of the safety of a food. FSANZ is satisfied with the data provided by the Applicant.

The recent publications by the Applicant (Ruebelt *et al*., 2006a; Ruebelt *et al*., 2006b; Ruebelt *et al*., 2006c) referred to in the INBI submission detail an analysis of the proteome of *Arabidopsis thaliana*, comparing naturally occurring *Arabidopsis* lines with a variety of transgenic lines.

However, the authors concluded 'on the basis of the changes detected for the proteins surveyed, the genetic modification of *Arabidopsis* using three different genes and three different promoters did not result in any phenotypic or seed proteome differences exceeding the natural variation other than the intended differences due to the introduction of the transgene'. Further comments were that 'Not much change was seen here that would inform a safety assessment.' Other studies have found similar results.

References:

Ruebelt, M. C., Leimgruber, N. K., Lipp, M., Reynolds, T. L., Nemeth, M. A., Astwood, J. D., Engel, K. H. and Jany, K. D. (2006a). Application of Two-Dimensional Gel Electrophoresis To Interrogate Alterations in the Proteome of Genetically Modified Crops. 1. Assessing Analytical Validation. J. *Agric. Food Chem.* 54:2154-2161.

Ruebelt, M. C., Lipp, M., Reynolds, T. L., Astwood, J. D., Engel, K. H. and Jany, K. D. (2006b). (2006b). Application of Two-Dimensional Gel Electrophoresis To Interrogate Alterations in the Proteome of Genetically Modified Crops. 2. Assessing Natural Variation. *J. Agric. Food Chem.* 54:2162-2168

Ruebelt, M. C., Lipp, M., Reynolds, T. L., Schmuke, J. J., Astwood, J. D., DellaPenna, D., Engel, K. H. and Jany, K. D. (2006c). Application of Two-Dimensional Gel Electrophoresis To Interrogate Alterations in the Proteome of Genetically Modified Crops. 3. Assessing Unintended Effects. *J. Agric. Food Chem*. 54:2169-2177.

R44: The Authority should know and report the detection level of the Western blots, and justify those detection levels if they are above the fmol range (Küster et al., 2001).

R45: The Authority should indicate how it has eliminated the possibility of post-translational modifications with molecules other than sugar.

The Western blots in this case are not intended to be quantitative. For a food safety assessment such a level of sensitivity is not necessary (fmol is 10^{-15} of a mole).

The issue of glycosylation analysis has been addressed above (e.g. response to R39 and R40). FSANZ is satisfied with the data submitted by the Applicant. It should be noted that the cDHDPS enzyme is functional in LY038: this indicates that minimal, if any, post-translational modifications have occurred.

R46: All previously un-notified changes in the protein profile of the plant compared to its non-modified parent should then be analysed for potential harmful affects on consumers.

See response to R43.

R47: The Authority should verify and then report whether the antiserum used for protein isolation was raised against *E. coli*-produced cDHDPS, *C. glutamicum* DHDPS, or *in planta*-produced cDHDPS (5.3.6.9). If the antiserum was not raised against the latter, then the Authority must confirm that the antisera will detect all *in planta*-produced isoforms detected by 2D gel electrophoresis and MS.

The antibody used was a polyclonal antibody and is expected to detect the range of epitopes that could be presented by cDHDPS regardless of whether it was raised against cDHDPS produced in *E. coli* or *in planta*. 2D gel electrophoresis and MS are not necessary.

R48: The Authority should confirm whether the antiserum was affinity purified and comment on how the purification might bias the reported results.

It is standard practice to affinity purify antisera to enrich for antibodies specific to the antigen. As the antigen in this case is a polypeptide, affinity purification would not affect to any significant extent the polyclonal nature of the antibody preparation. The overall specificity and sensitivity of the assay would not be adversely affected.

R49: The Authority should report how many exposures and how frequently goats were exposed to the antigen(s) and the antibody classes of the serum.

FSANZ does not consider that this level of detail is necessary. The specificity and functionality of the antibody preparation is clearly demonstrated.

R50: The Authority should report whether the antiserum affinity purified. If yes, the Applicant may have lost any antibodies that would bind to antigens unique to *in planta*-produced cDHDPS.

See response to R48.

R51: The Authority should address the possibility that other classes of antibodies could have masked epitopes from those classes used in the detection assay.

The antibody preparation used in the Western blot analysis clearly detects the cDHDPS protein produced from both *E. coli* and LY038 corn plants. This indicates that it is not masked by other classes of antibodies.

R52: The Authority should confirm that the antiserum was raised to *in planta*-produced protein(s) rather than raised against *E. coli*-produced cDHDPS or *C. glutamicum* produced cDHDPS.

See response to R47.

R53: The Authority should confirm that the goat anti-cDHDPS antiserum used is not affected by post-translational modification of cDHDPS, for example glycosylation, by demonstrating that the antisera will detect all *in planta*-produced isoforms detected by 2D gel electrophoresis and MS.

See response to R47. A polyclonal antibody preparation would be expected to detect a variety of potential isoforms. Further, the evidence indicates that cDHDPS is not glycosylated and there is no evidence to indicate that other post-translational modifications have occurred.

R54: The Authority should provide evidence that cDHDPS has no more propensity to form toxic aggregates when produced *in planta* than mDHDPS produced *in planta*.

The issue of the potential for cDHDPS to form aggregates was addressed at Draft Assessment. Concern was raised by INBI because amyloid fibrils are involved in a variety of medical conditions such as Alzheimer's and Parkinson's diseases.

However, these fibril aggregates are produced from endogenous proteins that have sustained mutations or have been misfolded, rather than from the consumption of particular dietary proteins.

The ability to form fibrils is not limited to those proteins involved in amyloidoses: it appears that any polypeptide can be induced to form fibrils under appropriate conditions *in vitro* (Chiti *et al*., 2000; Ellis and Pinheiro, 2002; Bucciantini *et al*., 2002). There is also some evidence that protein aggregates are inherently cytotoxic (Bucciantini *et al*., 2002). Therefore testing cDHDPS to determine if it forms cytotoxic fibrils would not provide useful information for a safety assessment of LY038 corn.

The cDHDPS protein is no more likely to form amyloid fibrils than any of the naturally occurring proteins in LY038 corn. Even in the event that cDHDPS aggregates form *in planta*, a series of improbable events would have to occur in order for cDHDPS fibrils to display cytotoxicity in human cells.

FSANZ is of the opinion that the studies submitted by the applicant demonstrate the safety of LY038 corn. The results of a study as suggested by INBI may be of academic interest but would not add significantly to the body of safety information.

R55: The Authority should provide evidence that proteins in the chloroplast of corn cells do not survive through digestion in humans, or cannot be taken up by gut cells.

R56: The Authority should provide evidence that all recombinant forms of cDHDPS are exclusively located in the chloroplast and not found in the ER, golgi or cytoplasm of plant cells at some concentration. If they are, then the Authority should provide reliable evidence that these forms do not survive through digestion in humans, or cannot be taken up by gut cells.

Whether cDHDPS is located in the amyloplast/plastid (corn grain does not contain chloroplasts) or elsewhere in the cell is not a food safety issue. Furthermore, many of the products likely to be produced from LY038 are highly processed products (e.g. corn oil or fructose syrup) and would not contain intact cells or organelles. cDHDPS is unlikely to be toxic or allergenic. It is not heat stable and is digested quickly.

R57: The Authority should provide evidence that transgenic cDHDPS aggregates do not form in the plant chloroplast or during cooking/processing of the whole food derived from the modified plant.

R58: If aggregates are detected, they Authority should provide evidence for their safety using established tissue culture assays for cytotoxicity and animal feeding studies.

See response to R.54

R59: The Authority should justify how it can assume the history of safe use of cDHDPS based on an extrapolation from the mDHDPS structure when there are profound differences in structure.

R60: The Authority should justify how it can assume the history of safe use of cDHDPS based on historical human consumption of natural cDHDPS.

The FSANZ safety assessment of cDHDPS is not based on an extrapolation from the mDHDPS structure or on a history of consumption of cDHDPS. It is based on the totality of evidence described in the safety assessment report, including an acute oral toxicity study of the protein in mice, and bioinformatics comparison to known protein toxins.

R61: Should the Authority recommend amendment of the Food Code to allow LY038, then it should impose quantitative restrictions on the levels of LY038 that may enter the human food supply to ensure that Applicant intentions are translated into responsible achievements should this material be approved for human food.

Having established that food from LY038 is as safe as food from conventional corn varieties, there is no regulatory justification for attaching restrictions or conditions of use once the food has been approved.

R62: The Authority should require a feeding study that meets the recommendations of Renwick (Renwick, 2004).

Renwick (2004) suggests that traditional toxicological studies should be conducted on single amino acids where the intakes may be extremely high (e.g. high dose supplements). As noted throughout the safety assessment report, the levels of lysine in LY038 grain are not high in comparison to other food sources of lysine (see table below).

Even using the high intake of corn by Mexican people, alluded to in the INBI submission, intakes of lysine from LY038 would not be particularly high. For example, daily consumption of 350 g LY038 corn (4800 ppm lysine) would supply approximately 1.68g lysine. This is equivalent to eating approximately 60g cheese or a similar amount of red meat. Corn intake in Australia and New Zealand is much lower (over 23 times less) than in Mexico.

From Draft Assessment Report Attachment 4:

Food	Lysine content (mg / 100 g food)[1]
LY038 grain	480
Control corn grain	320
Egg (hard boiled)	964
Fish	1500 - 2200
Red meat (beef & lamb)	1500 - 3300
Chicken	1700 - 2700
Cheese	700 - 2800
Lentils	489
Rolled oats	443
Broccoli	247

[1] Values are from ANZFA (1999) except for those for LY038 grain and control corn grain, which are from Appendix IV, page 224, of Monsanto's application to FSANZ and expressed on a dry weight basis.

R63: The Authority should request that the Applicant use the promising pig intestinal model (Baracos, 2004) for assessing amino acid toxicity.

See response to R62. This model has been put forward as a way of assessing amino acid toxicity when there is a high intake of a particular amino acid, particularly for individuals who are fed parentally and do not have intestinal and liver metabolism to regulate levels of amino acids in the blood. Consumption of high lysine corn will not result in high dietary intakes of lysine so a study of the type reported by Baracos (2004) is not relevant to the safety assessment of LY038.

Reference:

Baracos VE (2004). Animal models of amino acid metabolism: A focus on the intestine. *J. Nutr.* **134**:217-230.

R64: The Authority should make the 3-month rat feeding study available to the independent scientific community for evaluation before recommending to Council that the food code be amended to include LY038 corn.

The Applicant has provided appropriate evidence that the 3-month feeding study in rats conducted with LY038 corn is a trade secret relating to food and requested that it remain confidential. Following an evaluation of this evidence against the criteria in the FSANZ Act, the request was approved. FSANZ has fully assessed the study, a summary of which is provided in the Safety Assessment Report, however the full study is not a public document.

The feeding study forms only one part of the evidence supporting the safety of LY038 corn. The final decision on the safety of LY038 corn was based on the totality of the evidence as described in the Safety Assessment Report.

R65: The specific activity data is inappropriate for drawing conclusions of identity or functional similarity. Better measures for functional similarity, such as K_m and V_{max}, should be provided.

Information on the specific activity of a novel protein such as cDHDPS is not required by FSANZ to establish food safety, or to determine similarities between the LY038 and *E. coli* produced cDHDPS protein. Where an Applicant has provided such data, it can be included in the safety assessment report for information, and adds minimally to the overall picture of cDHDPS.

R66: The Authority should draw a recommendation based in part on feeding studies using the whole food (grain of transgenic plants and cooked products that would form a representation of how the food was to be consumed by people).

See response to R14 and R18.

R67: The studies should be conducted using animal models that are most appropriate for identifying harms relevant to people. Long-term (lifetime) studies should be included because high lysine corn is also high free lysine, saccharopine, α-aminoadipic acid, cadaverine and pipecolic acid corn. The Authority should report on chronic effects, evidence of carcinogens and co-carcinogens (AGEs have been linked to cancer Heijst et al., 2005), and proteins that are capable of forming aggregates.

No structural analysis alone will predict the effect of context on an enzyme or its potential to produce unanticipated products in a novel context. Therefore, structural analyses equating *E. coli*- and *in planta*-produced cDHDPS cannot substitute for the use of *in planta*-produced cDHDPS in all biochemical and feeding experiments (NZIGE Submission section 5.3.7.2).

See responses to R14-16, R18 and R54.

R68: The Authority should report how both dietary and airborne allergens in LY038 were excluded by experimental tests conducted on animals previously fed the whole food derived from LY038.

The rat feeding study using LY038 corn grain was not intended to assess the potential allergenicity of the food. No validated animal or other model exists that can accurately predict the allergenicity of proteins in food. Instead, FSANZ applies an integrated, stepwise, case-by-case approach, as described in the Codex guideline (CAC/GL 45-2003), to assess the potential allergenicity of any novel proteins. The Applicant has fully addressed all of the data requirements for allergenicity assessment and FSANZ is satisfied, on the basis of the evidence provided, that cDHDPS is unlikely to be a food allergen.

R69: For allergen identification, we are more concerned with false negatives than false positives. Thus we ask the Authority to review the bioinformatics data using the parameters set by FAO/WHO.

The 2001 FAO/WHO expert consultation on the evaluation of allergenicity of GM foods suggested that a search for identity over 6 contiguous amino acids be performed. However, the committee acknowledged that identity over 6 amino acids has an appreciable risk of occurring by chance and it should therefore be performed in conjunction with other analyses including homology analysis across 80 amino acids and verification of cross-reactivity with suitable antibodies (either animal or human).

This advice was taken into account by the Codex *Ad Hoc* Intergovernmental Task Force for Foods Derived from Biotechnology in establishing the Codex guideline, which states that sequence homology searches should be done. The Codex guideline states that strategies, such as stepwise contiguous identical amino acid segment searches may also be performed for identifying sequences that may represent linear epitopes. The guideline then states that the size of the contiguous amino acid search should be based on a scientifically justified rationale in order to minimise the potential for false negative or false positive results. Although the Codex Task Force recognised the 2001 FAO/WHO expert consultation suggested moving from 8 to 6 identical amino acid segment searches, this was not adopted in the Codex guideline, which states that the smaller the peptide sequence used, the greater the likelihood of identifying false positives, and the larger the peptide sequence, the greater the likelihood for false negatives.

In this assessment, FSANZ accepted the use of search criteria using 8 contiguous amino acids and is satisfied that the analyses performed by the Applicant are sufficient to conclude that the novel protein, cDHDPS, is unlikely to be a food allergen.

R70: The Authority should report the results of a bioinformatic analysis using the actual sequence of in planta-produced recombinant cDHDPS.

See response to R42.

R71: Whereas there may be virtue in establishing a standard, as the industry-led groups in the Thomas *et al.* study did, it remains unclear why the FAO/WHO protocol is not the standard nor why reproducibility is a greater virtue than using a pH relevant to conditions in the stomach during a meal, such as pH 4-5 (Schmidt et al., 1995, Thomas et al., 2004). The Authority should require results to the Thomas *et al.* industry-preferred standard *and* the FAO/WHO standard.

FSANZ is satisfied with the results of the study provided by the Applicant and does not agree that it is necessary to request two different digestibility studies. This issue was addressed at Draft Assessment as follows:

The Applicant conducted an *in vitro* digestibility study on the novel protein present in LY038 corn, cDHDPS, using a standardised protocol that has been shown to distinguish known allergens from proteins known not to be allergenic (Thomas *et al*., 2004). This protocol is not intended to be an exact replica of conditions *in vivo*, but rather is used to compare the test protein to known allergens under the same conditions.

The NZIGE object to the use of this protocol because the ratio of pepsin to protein is higher than would occur naturally in the human stomach and gastrointestinal tract. 10U of pepsin were used for every µg of test protein (2.64:1 ratio based on weight). Although *in vivo* protein levels will almost always exceed those of pepsin (Taylor, 2003), a standardised pepsin resistance assay is needed. For this reason the Applicant has used a protocol that has been shown to distinguish *in vitro* known allergens from non-allergens.

The recommendations of the WHO/FAO paper (2001) does not specify pepsin activity, but recommends an amount of pepsin based on weight. However, in reactions of this kind, enzyme activity is more relevant to the outcome than enzyme weight and for this reason, the protocol used by Thomas *et al*. (2004) is considered by FSANZ to be appropriate for assessing relative digestibility.

R72: The Applicant should report digestibility measurements after processing/cooking of material from whole food.

FSANZ does not consider such analyses to be necessary or useful. Corn products have a long history of use as a food and as such are readily digestible. It is reasonable to expect similar results for LY038 corn products. See responses to R14-16.

R73: The Authority should require, at the very minimum, results of the digestion studies using a surrogate source of protein verified to represent all post-translationally modified forms of the protein in whole food, including after cooking and processing. For this, the Authority will have to address our call for using 2D gel electrophoresis and MS to identify all isomers produced in plants.

There is no evidence to suggest that cDHDPS is post-translationally modified. Glycosylation analysis does not indicate that it is glycosylated *in planta* and the enzyme is clearly functional. Digestibility studies such as these would not be feasible and FSANZ does not consider that the results would contribute in any significant way to the safety assessment of food derived from LY038.

R74: The Authority should provide the results of blood tests and data on organ weights and visual observations.

Referring to MSL-18883 (the broiler chicken feeding study), blood analysis and organ weights are not performed routinely as part of a feeding study. The primary purpose of this study was to assess the nutritional value of LY038 corn when used as animal feed; in this case, the ability of LY038 corn to support typical growth and well-being in rapidly growing chickens.

Analyses conducted as part of this study included: bodyweight and bodyweight gain; feed intake; feed conversion efficiency; carcass yield; meat composition; fat pad measurements; and breast and thigh meat quality. All of these parameters (vigorous growth and carcass characteristics) are key indicators of the nutritional adequacy of the food.

Although blood analysis, organ weights and a variety of other parameters are routinely performed in toxicological studies, feeding studies test other factors that are linked to the nutritional characteristics of the food. As such, it is not appropriate to expect a similar set of results from these different types of studies. Unlike traditional toxicological tests that are used for drugs, a feeding study cannot establish a dose level at which adverse effects occur and, from that, derive a safe level of food intake. Nutritional imbalances, and other confounding factors, occur when an animal is exclusively fed high levels of a single food. It is generally agreed that it is therefore not appropriate to apply toxicological studies to whole foods because of these methodological issues.

R75: The Authority should seek a satisfactory re-evaluation of the effects on chicks in the first 21 days.

FSANZ has evaluated study MSL-18883 and found no meaningful difference in the growth of broilers fed LY038 compared to conventional corn diets supplemented with lysine over the first 21 days of the study. There was no significant difference between LY038-fed chicks and chicks fed conventional corn diets over this period in either the pen weight, average bird weight, pen weight gain or average bird weight gain. Differences observed in feed efficiency over days 0 – 21 are not considered biologically relevant due to the relatively small feed consumption during this period. A difference was not observed in comparisons across days 22-42, nor across days 0-42.

R76: The Authority, at the very least, should seek a feeding trial using LY038 rather than a mix of transgenic strains that dilutes LY038.

The corn used in the rat feeding study was tested and confirmed to be >99.25% LY038 grain, therefore FSANZ does not agree that LY038 was ‘diluted’ in this study.

However, testing indicated that <21% of the grain also contained the MON810 trait, suggesting that a minor proportion of the grain was LY038 x MON810. MON810 is an approved variety of GM corn and its presence is not anticipated to affect the outcome of the study.

R77: We agree with the Authority that high-lysine corn is a significantly changed product. We therefore recommend that properly conducted feeding trials be made available for review by the Authority and, if possible, the public. These trials will use animals suitable for gauging food safety in humans (i.e., not chickens), possibly pigs, and will use cooked and processed whole foods.

See response to R18 and R20. An acute toxicity study with the purified novel protein and a subchronic study in rats with whole LY038 have been performed. Based on the results of these and other studies, for example biochemical studies, FSANZ does not consider additional animal studies to be necessary to establish the safety of the food.

R78: One, possibly several, genes in LY038 are likely have been affected by the transformation process to explain accumulation of lysine in the seed. As recommended in CAC/GL 45-2003(33-D), the Authority should be able to explain how LY038 accumulates these levels of free lysine in grain and demonstrate that the mechanism would be exactly the same in all hybrids.

The mechanism by which LY038 corn has increased levels of lysine is explained in the safety assessment report. As the modification is stably expressed and inherited from one generation to the next in the predicted fashion, there is no reason to expect that the mechanism would be different in hybrid lines. Although expression patterns of other genes may well be altered in LY038 as a consequence of higher biosynthesis of lysine, the compositional analyses address this issue.

R79: Should the Authority recommend an amendment to the Food Code, then the Authority should impose a condition in Column 2 of the Table to Clause 2 of Standard 1.5.2 that limits this approval to LY038 without extension to hybrid lines derived from LY038. All hybrids, whether between LY038 and an unmodified line or another approved modified line, must in this case be treated as a new organism requiring a full safety evaluation. If it cannot do this, then it should not recommend amendment of the Food Code.

See responses to R36 and R37. As explained by FSANZ in the response to the NZIGE submission at Draft Assessment, food from a hybrid plant line that has been created by conventional breeding methods does not warrant a separate pre-market safety assessment, provided that the parental GM plant lines are considered safe. The food safety risks posed by conventional breeding programs using GM lines are no different to those arising from the conventional breeding of non-GM plants. Unintended changes may occur as a result of conventional breeding, however the products of conventional breeding are regarded as having a history of safe use and are not regulated by FSANZ.

R80: We ask the Authority to detail its position with reference to developments at the international level.

R81: If the Authority has requested details from the Applicant on its post-market surveillance plans, we ask for these to be released and for the Authority to publish its evaluation. If the Authority has not requested these details, we recommend that they are requested now. If the Authority does not feel obliged to do so, we ask for an explanation as to why.

GM food products are not permitted on the market if any question associated with public health and safety is left unanswered during the pre-market safety assessment. Such an assessment already provides assurance that a GM food is as safe as its conventional counterpart. On this basis, long-term effects specifically attributable to GM foods would not reasonably be expected to occur.

FSANZ does recognise that a form of post market surveillance may be desirable for some GM foods developed with specific nutritionally enhanced characteristics, where effects in the population would be expected. In the case of high lysine corn however, the levels of lysine in LY038 are not sufficient to affect the nutritional status of the population. In addition, given its primary purpose as animal feed, post-market monitoring of LY038 corn is not warranted.

R82: FSANZ should reconsider its statement, made in relation to this hypothetical benefit to consumers noted above, that: 'As food from LY038 corn has been found to be as safe as food from other varieties of corn, option 1 is likely to be inconsistent with Australia and New Zealand's WTO obligations.' We have demonstrated that this is an unreasonable conclusion to draw, given the scientific concerns we have listed as well as the fact that Codex Alimentarius and WHO recommended practices, which are acceptable under WTO, would require more stringent scientific scrutiny.

R83: The Authority states that 'Government: Potential impact if considered inconsistent with WTO obligations but impact would be in terms of trade policy rather than in government revenue' and 'Industry: Potential longer-term impact - any successful WTO challenge has the potential to impact adversely on food industry'. It should clarify the weighting given to WTO considerations and the relative cost attributed to this in the draft decision.

R84: FSANZ should reconsider its Impact Analysis and its decision from the perspective of the full range of eventualities its decision makes possible, in particular the various ways in which LY038 may inadvertently or deliberately be introduced into the food supply as well as the issues of prevalence, hybrids, dietary restrictions, and distribution of costs and benefits that we have noted.

R85: We ask FSANZ to clarify: in its decision-making, is it considering potential cost to government, or not? And if so, how can it assign weight to monetary costs without attempting to quantify them? And does it give equal consideration to costs of each option (approval and non-approval), e.g., to the (certain) costs of monitoring as much as to the (speculative) costs of responding to illegal contamination?

R86: If FSANZ is not considering potential cost to government, we ask that it explain the reasoning behind including the Impact Statement relating to government monitoring resources. If FSANZ *is* considering potential cost to government (as indicated by a number of statements in the DAR), we ask again that it provide evidence that the cost to government of monitoring for the presence of LY038 in food will be low.

R87: In line with INBI's previous submission, FSANZ should also provide evidence that the monitoring and labelling cost to industry will be low.

FSANZ has assessed a comprehensive package of information on LY038 corn and has found no food safety concerns. In addition, the concerns raised by INBI have been carefully considered, cited scientific references have been examined and the relevance of alternative data sets has been analysed in the context of a food safety assessment. FSANZ reaffirms the conclusion that food from high lysine corn is as safe as food from conventional varieties of corn. The impact analysis has been made on the basis of this conclusion, and is therefore not complicated by considerations that the food should not enter the market, or that it should be required to comply with special conditions of use, or targeted for post-market surveillance.

The impact analysis recognises that costs to government, industry and consumers may result from the approval of food from LY038, as with other food approvals. However, it also recognises that the alternative option incurs costs for these sectors, although the impact across sectors may vary. Given the favourable safety assessment, approval of food from high lysine corn has been identified as the preferred option.

R88: Should FSANZ recommend amending the Code for the event in LY038, then we recommend that threshold criteria be established in Column 2 of the Table to clause 2 of Standard 1.5.2 indicating below which levels and frequency of contamination, and range of contaminated products, LY038 events would be seen as inadvertently contaminating the human food supply and what the consequences would be for contamination above these thresholds.

Refer to R61 and R 82-87. As stated in previous responses, there is no basis for imposing restrictions or caveats on food from LY038 corn, should it be approved.

R89: Should FSANZ recommend amending the Code for the event in LY038, then we recommend that only certain existing varieties and hybrids be allowed (those that have met stringent testing as described above and in our first submission) and not extend to other varieties with the same event.

See response to R79, and also R 82-87.

R90: FSANZ should explain how it derived a conclusion of 'net benefit to food producers and consumers' from the analysis presented.

R91: In light of the Authority's commitment to 'increased accountability and transparency in decision making' (Australia New Zealand Food Authority, 2001), FSANZ should explicate for the public the process it uses to move from impact analysis to preferred option, including an explanation of how various factors have been weighted and how public input has been taken into account.

See response to R82-87.

R92: The Authority should clarify whether it contracted external parties to review A549.

The safety of LY038 was assessed by FSANZ scientific staff who are experienced in conducting food risk assessments. These same members of staff have professional backgrounds in the following disciplines: biochemistry, plant molecular biology, human physiology, toxicology and allergenicity assessment and are considered well qualified to carry out safety assessments on GM foods.

As has become normal practice for FSANZ in recent years, following completion of the Draft Assessment Report, FSANZ sought peer review of the safety assessment from two independent external scientists with relevant expertise. In this case, the reviewers support the conclusions of the safety assessment. Their comments, which will be part of the Final Assessment Report, have been addressed through minor modifications to the safety assessment.

R93: FSANZ should explain the process it used to identify an independent reviewer for INBI's IAR submission, including the criteria it used to determine the reviewer's independence.

FSANZ has had a policy of engagement of external experts for a number of years, starting with the Fellows Program in 2000. This approach is not restricted specifically to the safety assessment of GM foods, but extends to a broad range of food regulatory matters including health claims, iodine and folate fortification, allergenicity and food intolerance, and the recent major projects to develop primary production standards. It was recognised that peer review of our scientific risk assessments was an effective method of ensuring that the FSANZ Board is provided with the best possible advice when making food regulatory decisions. Moreover, it is normal practice for scientific papers to undergo review before publication, and FSANZ considers that seeking external comments on our assessments is compatible with this process.

In relation to external reviews of GM food assessments in general, FSANZ developed a list of scientific advisors some years ago and has used the list periodically to seek one or two reviews of Applications dealing with a novel gene or modification that has not previously been assessed. The list includes scientists working in New Zealand, South Australia, Western Australia, Victoria, Queensland and the Australian Capital Territory. Experts are generally approached on the basis of their academic background and knowledge of certain commodities, as well as their publication record.

R94: In considering the comments of the independent reviewer, FSANZ should take into account the fact that the reviewer's conclusions were based on differences of judgment, rather than findings of scientific error.

Any comments received, whether through the public submission process or from commissioned reviews of FSANZ's work, are scrutinised for their scientific objectivity. Wherever appropriate, FSANZ uses these comments and suggestions to increase the rigour of the assessment process to assist with the regulatory decision.

In food regulatory environments around the world, the safety assessment of GM foods is appropriately focused on agreed principles and obtaining relevant scientific information using a suite of current validated methods. This should not be interpreted to mean that the approach to the assessment of GM foods is fixed, but rather that the value of certain emerging methodologies in assessing food safety risks is by no means resolved or established.

A technical capability in one particular field does not necessarily translate into other areas of science.

In this context, while INBI may raise some academic points of interest, these are not necessarily relevant to the current process of assessment and arguably are not even specific for GM foods but could apply equally to foods from non-GM sources. Overall, INBI's approach to the safety assessment is impractical and its requirements for data are not commensurate with the level of risk posed by the foods. The requirement for certainty at all levels of the assessment is scientifically unattainable.

One of the strengths of the current approach used by FSANZ and other regulators is the flexibility afforded by the guidelines, consensus documents, and case-by-case management of issues, which can accommodate the idiosyncrasies of each GM food. FSANZ sees strength in using a process that reflects an international consensus based on a combined knowledge and expertise in assessing food-related risks.