2-06
22 March 2006

DRAFT ASSESSMENT REPORT

APPLICATION A549

FOOD DERIVED FROM HIGH LYSINE CORN LY038

DEADLINE FOR PUBLIC SUBMISSIONS: 6pm (Canberra time) 3 May 2006
SUBMISSIONS RECEIVED AFTER THIS DEADLINE
WILL NOT BE CONSIDERED
(See 'Invitation for Public Submissions' for details)

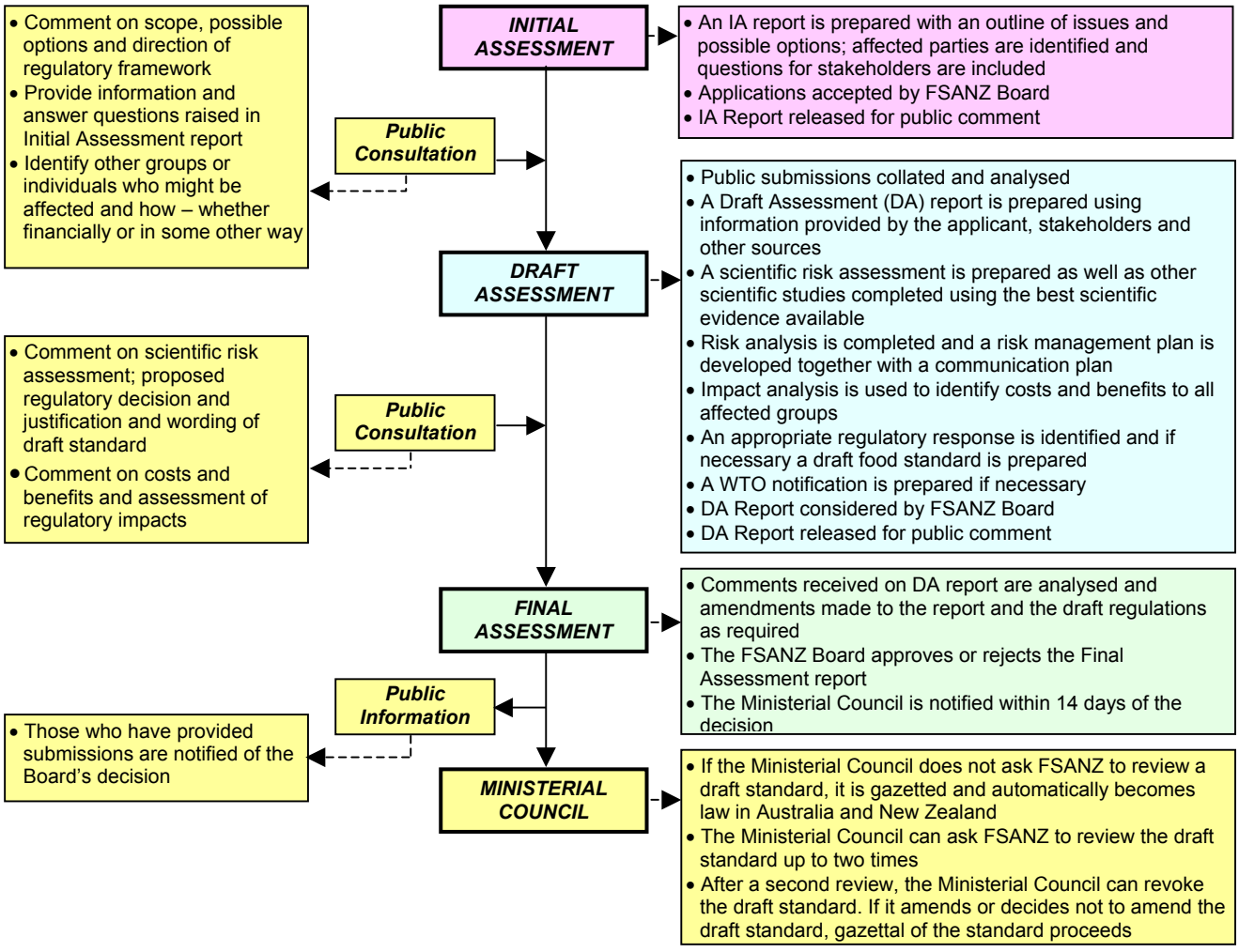
FOOD STANDARDS AUSTRALIA NEW ZEALAND (FSANZ)

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

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

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

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



INVITATION FOR PUBLIC SUBMISSIONS

FSANZ has prepared a Draft Assessment Report of Application A549; and prepared a draft variation to the *Australia New Zealand Food Standards Code* (the Code).

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

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

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

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

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

Food Standards Australia New Zealand
PO Box 10559
The Terrace WELLINGTON 6036
NEW ZEALAND
Tel (04) 473 9942
www.foodstandards.govt.nz

Submissions need to be received by FSANZ by 6pm (Canberra time) 3 May 2006.

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

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

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

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Executive Summary and Statement of Reasons

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 allows the accumulation of lysine in the corn grain. Corn line LY038 is intended specifically for animal feed, however it is possible that a small percentage may enter the human food supply. Corn line LY038 does not contain any additional novel genes.

If approved, food from corn line LY038 may enter Australia and New Zealand as imported products.

Public submissions are now invited on this Draft Assessment Report. Comments are specifically requested on the scientific aspects of this Application, in particular, information relevant to the safety assessment of food from corn line LY038.

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 nutritional 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 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

If approved, 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 products 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 it potentially offers benefits to all sectors with little associated cost. The proposed amendment to the Code, giving approval to food from corn line LY038, is therefore considered of net benefit to both food producers and consumers.

Consultation

FSANZ made an Initial Assessment of this Application and called for submissions on 15 December 2004. The closing date for submissions was 9 February 2005. Two hundred submissions were received. A summary of these is in Attachment 3. Issues raised by the submitters in relation to this Application are discussed in Section 5.4.

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 recommended on the basis of the available scientific evidence for the following reasons:

- the safety assessment did not identify any public health and safety concerns associated with the genetic modification used to produce corn line 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;

- 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.

It is proposed that the draft variation 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.

A Draft Assessment of the Application, including a detailed safety assessment of food derived from corn line LY038, has been completed and FSANZ has prepared a draft variation to Standard 1.5.2 of the Code (see Attachment 1). Public comment is now being sought to assist in the Final Assessment of the Application.

2. Regulatory Problem

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

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 Draft 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

GM corn plants have been developed by the Applicant 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 LY038 in our food supply would be in imported products. The types of food products that might be likely to contain 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.

Applications have been made to use corn line LY038 for food and feed in the United States, Canada, Japan, the European Union and Argentina. No approvals have been granted yet.

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

An assessment of the nutritional impact LY038 corn might have on the human food supply has been made. 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 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.

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, little LY038 will be entering the food supply, mostly in the form of processed products (e.g. corn syrup) that contain negligible amounts of protein.

The Applicant has provided information comparing LY038 corn to a closely related control corn crop, LY038(-), both grown in the same location. 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.

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 shows that the plant lysine metabolic by-products, cadaverine 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 and also where the food has altered characteristics. If approved, 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 products 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 Studies conducted to support the safety of LY038 corn

A number of 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.

5.4.1.1 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.

5.4.2 Safety of LY038 corn for human consumption

A number of submitters expressed the concern that as LY038 corn is intended only for animal feed, that the safety assessment of this product by FSANZ might be less rigorous than the assessment of a GM product intended for human consumption.

5.4.2.1 FSANZ Response

FSANZ has responsibility for the safety of food sold in Australia and New Zealand. FSANZ is assessing LY038 corn as if it were intended to be consumed by humans. The safety assessment conducted on LY038 is as rigorous and thorough as for any GM food product, and assumes that if approved, corn from line LY038 could be routinely entering the food supply and not present just as an occasional inadvertent ingredient. Although this is unlikely, LY038 corn must be shown to be as safe as other varieties of corn currently available if it is to be approved by FSANZ.

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

5.4.3 New Zealand Institute of Gene Ecology (NZIGE) Submission

The NZIGE reviewed all the data submitted to FSANZ in support of this application and compiled a detailed submission. Twenty recommendations on A549 were made to FSANZ. Points raised in the recommendations include:

- it is possible that changes in protein expression may occur in the plant due to expression of the novel protein in LY038 (cDHDPS) and that this possibility should be assessed by comparative 2D electrophoresis and mass spectrometry;
- long-term (lifetime) animal feeding trials should be conducted, a 6-month toxicity study should be conducted and feeding studies in humans should also be performed
- analysis should be done on Maillard reaction products;
- the potential for aggregation of cDHDPS should be assessed;
- micro-array analysis should be done to detect changes in the transcriptome of LY038;
- inserted DNA should be analysed for putative mammalian transcription factor binding motifs;
- metabolomic analysis should be performed;
- the digestibility assay should be performed using a protocol consistent with the FAO/WHO (2001) and the recommendations of Pusztai et al (2003);
- the compositional analysis should be extended to more different lines of conventional corn;
- the broiler feeding study should be dismissed for purposes of assessing safety of LY038;
- animal experiments should monitor cholesterol levels and changes in phospholipids;
- a post-launch monitoring strategy should be submitted by the applicant;
- FSANZ should clarify its jurisdiction with regard to the approval of a food intended for use only as an animal feed;
- FSANZ should not allow the intention of the Applicant to segregate LY038 from the food supply to influence the rigor of the assessment process;
- FSANZ should provide evidence for the costs and benefits mentioned in the regulatory impact statement for both options one and two;
- FSANZ should not approve any hybrid corn lines derived from LY038; and
- FSANZ should consider New Zealand's obligations under the Cartagena Protocol on Biosafety.

5.4.3.1 FSANZ response

The recommendations made by the NZIGE and FSANZ's response to these are presented in Attachment 4 to this report. These recommendations have been taken into consideration in this Draft Assessment Report and attached Safety Assessment where appropriate.

5.4. Issues relating to the impact assessment presented in the Initial Assessment report

The South Australian Department of Health and the NZIGE noted that some of the impacts relating to the two options presented in the Initial Assessment Report may not be relevant considerations given that FSANZ has jurisdiction over food but not animal feed and also that LY038 corn is not to be grown in Australia and approval would therefore have no effect on Australian and New Zealand corn growers.

5.3.4.1 FSANZ Response

The impact analysis for each of the two options (approval of LY038 corn or no approval of this corn) has been reassessed and is presented below in Section 7.

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 initial assessment by FSANZ of the costs and benefits of the two regulatory options identified so far. This is based on information supplied by the Applicant and experience FSANZ has gained from consideration of previous applications relating to GM foods. Your comments are also invited on the costs and benefits identified for the options below.

7.2.1 Option 1

Consumers: Benefit to consumers if there is a public health and safety concern.

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

Government: No immediate impact.

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

Industry: No immediate impact.

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

7.2.2 Option 2

Consumers: No direct impact.

The amount of LY038 corn entering the food supply is likely to be low so the cost to consumers wishing to avoid GM food by a potential restriction of choice of products, or increased prices for non-GM food is likely to be low.

Government: No direct impact.

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.

Industry: No direct impact.

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 approval of this line may prevent problems in the future if LY038 were to accidentally 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 and a summary of these is included in **Attachment 3** to this Report.

FSANZ has taken issues raised by submitters into consideration in the Draft Assessment of Application A549. These issues have been addressed in Section 5.4.

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 Standards 1.5.2 of 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 will be recommending to the agencies responsible that the WTO be notified under the Sanitary and Phytosanitary Measure (SPS) Agreement, in order to enable other member nations to comment on the proposed changes to standards that may have a significant impact on them.

9. Conclusion and Recommendation

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 recommended on the basis of the available scientific information 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;

- 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 the Code is consistent with the section 10 objectives of the FSANZ Act and the regulatory impact assessment.

The proposed draft variation is provided in **Attachment 1**.

10. Implementation and review

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

ATTACHMENTS

1. Draft variation or standard to the *Australia New Zealand Food Standards Code*
2. Safety Assessment Report
3. Summary of issues raised in public submissions
4. FSANZ Response to New Zealand Institute of Gene Ecology Submission

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 were used to demonstrate 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. Glycosylation analysis showed that cDHDPS is not glycosylated in LY038.

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 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 LY038 in our food supply would be in imported products. The types of food products that might be likely to contain 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.

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

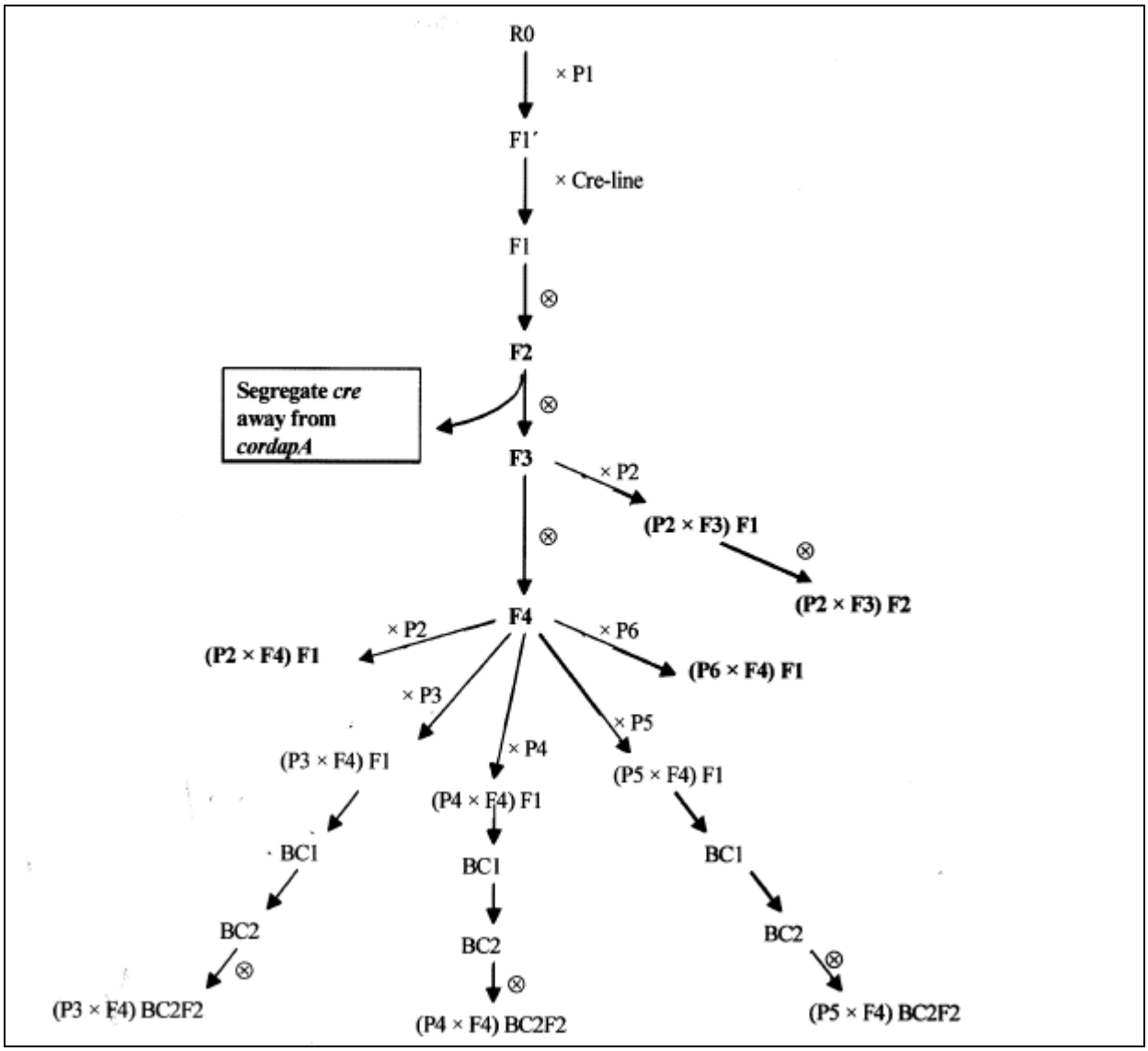
3.1 Method used in the genetic modification

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.

Through extensive PCR analysis of subsequent maize breeding progeny, the *cre* gene was segregated away from the *cordapA* gene such that F3 progeny containing only the *cordapA* gene cassette and lacking both the *nptII* and *cre* gene cassettes were identified and designated as Lysine maize LY038. The absence of the *cre* and *nptII* gene cassettes in corn LY038 was demonstrated by event specific PCR analyses and extensive Southern blot analyses.

Figure 1: Diagrammatic representation of LY038 corn breeding tree



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, demonstrated that there is only one copy of the *cordapA* gene and associated regulatory elements present in the genome at a single locus.

The *nptII* and *cre* gene cassettes and plasmid backbone sequence are absent. The negative segregrant, LY038(-), was shown to lack any novel DNA.

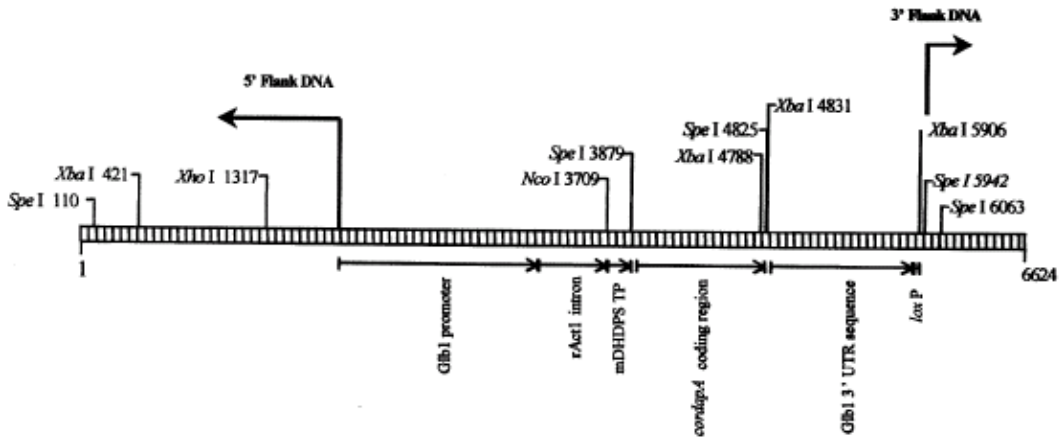


Figure 2: Linear map of the insert and adjacent DNA flanking the insert in LY038 corn

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.

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 6 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.

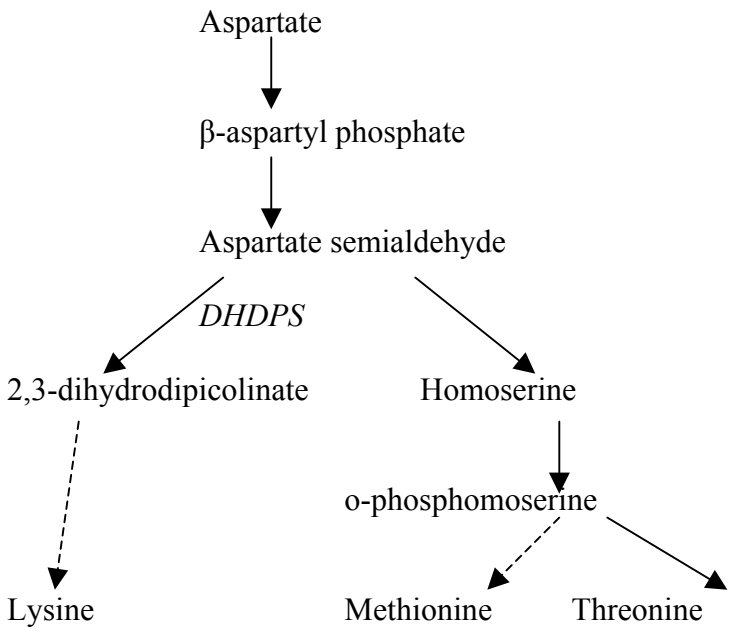


Figure 3: Aspartate pathway of lysine biosynthesis

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 33kDa. 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 is anticipated that it may 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.

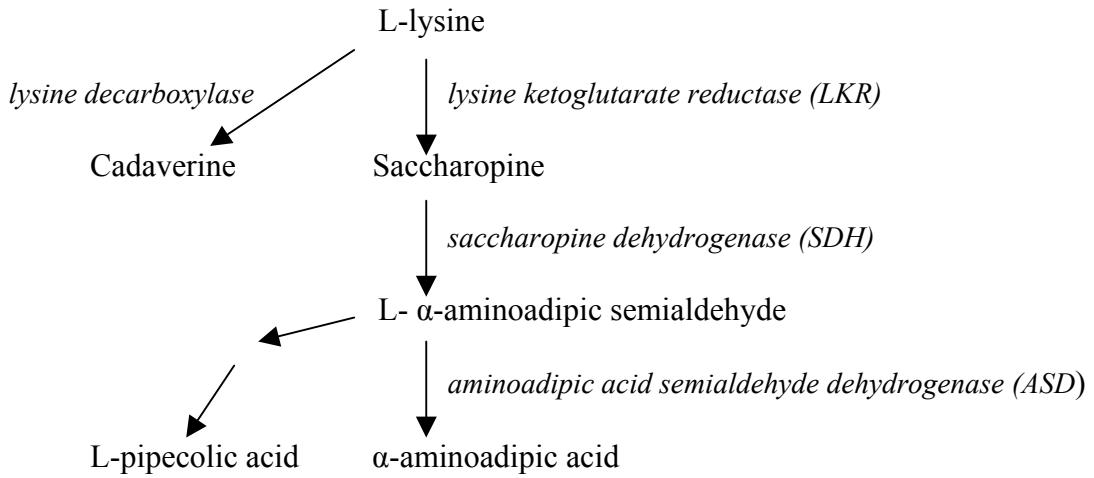


Figure 4: Enzymatic catabolism of lysine in seed (adapted from Galili, 1995)

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 has been used as a treatment for the herpes virus. 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 demonstrated 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

Potential dietary exposure to novel protein

The highest level of expression of cDHDPS in the grain of LY038 based on the expression data above was 26 ng/g dry weight. However, the actual exposure to this protein in the diet is expected to be much lower than this. LY038 corn is intended strictly for use as an animal feed for poultry and possibly pigs. As such, LY038 corn will be subjected to identity preservation methods to segregate this nutritionally enhanced animal feed from conventional commodity grain to ensure the recovery of the commercial value of this product. Even at peak market penetration, the Applicant expects that this crop will represent less that 7% of the field corn grown in the U.S.A. and because of the identity preservation methods that will be used to segregate this product from conventional grain, it is unlikely that more the 5% on average LY038 would be inadvertently co-mingled with conventional corn and enter the human food supply. Much less than this would be expected to reach Australia or New Zealand, and much of this would be as processed products such as high fructose corn syrup that contains little or no protein.

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 survived the two-week observation period. No clinical signs were observed during the study. There were no significant differences in body weight or body weight changes between the test and control groups. 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 as the development of cysts is unlikely to not occur in 14 days.

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

A possible concern is that new proteins introduced into food will 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 proteins 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 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.

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 polyacrylimide 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 methionine 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.

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 also 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 highly unlikely event that any of the alternative reading frames 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, is rapidly digested and there will be very limited exposure to this protein.

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 are discussed below.

History of consumption of 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 a cause for concern. 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.

In addition to exposure to these lysine metabolites through the consumption of common foods, animals and humans are exposed to both of these metabolites as products of normal endogenous lysine metabolism.

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 feel 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	Yes	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 composition, terminal clinical pathology tests, organ weight measurement, and 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.

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ATTACHMENT 3

Summary of First Round of Public Submissions

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.
- Recommend that the safety assessment takes into consideration the potentially very low dietary exposure to this product.
- Believe 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) (-)

- Agree that the issues raised in the IAR need to be considered.
- Ask 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)

- Oppose 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)

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

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

- Oppose 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)

- Oppose GM food

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

- Oppose 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)

- Support NZIGE submission.
- Oppose 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
- Support 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) (-)

- Support 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. J. R Collins

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

299. 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

ATTACHMENT 4

Response to New Zealand Institute of Gene Ecology submission

At Initial Assessment, the New Zealand Institute of Gene Ecology (NZIGE) reviewed all the data submitted to FSANZ by Monsanto in support of this application and compiled a detailed submission, outlining a number of areas where it believes there are deficiencies in the safety data. Since Initial Assessment, FSANZ has reviewed all the data (including additional data submitted by the applicant at the request of FSANZ) and completed a safety assessment on food derived from LY038 corn (at Attachment 2 to the Draft Assessment).

The NZIGE made 20 recommendations on Application A549. FSANZ has addressed each of these recommendations below.

R1: In order to make an assessment of the changes in protein expression that occur within the plant due to expression of cDHDPS, the Applicant should undertake a routine proteomic analysis (by comparative 2D electrophoresis and mass spectrophotometric analysis of relevant spots) of lysates from whole plant cells and demonstrate that the only change is expression of the inserted gene. Single dimension protein gels of whole plant extracts at various stages of purification should also be supplied, in order to authenticate the purification of cDHDPS from plant tissue. Presentation of only the purified protein is unacceptable.

Although general profiling techniques such as proteome analysis can be valuable research tools, a major challenge is in determining whether observed differences between two plants are distinguishable from natural variation, and if so, their relevance to food safety. For such techniques to be of use for the purpose of regulation they must be validated and the baseline range of natural variation clearly established (ILSI, 2004). As the feasibility of applying such techniques to food safety assessment is still being investigated, it is unlikely that information from proteome analysis would add to the safety assessment of GM foods.

FSANZ has evaluated the molecular analysis of the inserted DNA in the genome of LY038 corn, which shows that only one copy of the gene encoding cDHDPS is present. No other novel proteins are produced in LY038. A full characterisation of this protein was done as part of the safety assessment of LY038 and is described in Section 4 of Attachment 2 of the Draft Safety Assessment Report. The data provided was comprehensive and included N-terminal sequencing, molecular weight determination, immunoreactivity, glycosylation analysis, peptide mass fingerprinting and matrix assisted laser desorption ionisation time of flight (MALDI-TOF) mass spectrometry. Further information such as suggested in this recommendation would not add to characterisation of cDHDPS and therefore has not been requested by FSANZ.

R2: The Authority should require data from long term (lifetime) animal feeding trials to capture chronic effects, detect carcinogens and co-carcinogens, and proteins that are capable of forming amyloid fibrils.

It is unclear whether this recommendation refers to feeding studies with the purified protein or with the whole food. Long term feeding studies in animals are of limited value for determining the safety of whole foods, including GM food.

The safety assessment of a GM food relies largely on a comparison with its conventional counterpart. This is discussed further under recommendation 12.

The biochemical components identified in LY038 corn are the normal constituents of conventional corn with the exception of the cDHDPS protein. This protein is structurally and functionally related to an endogenous DHDPS protein. As with any whole food, incorporation of LY038 corn into the diet of animals at high levels and over long periods of time is likely to cause physiological and/or biochemical changes due to nutrient imbalances, rather than any specific toxicity. In terms of the contribution of diet to cancer risk, over the long term a balanced diet of nutritious foods is probably more important to health that the level of intake of any one food.

There is no scientific justification for FSANZ to request lifetime carcinogenicity studies with LY038 corn.

Amyloid fibrils are discussed under recommendation 4.

R3: The Authority should request an analysis of Maillard reaction products or other glycotoxins that could arise from cooking or processing of LY038 corn.

This recommendation is based on the concern that any increase in lysine in food may lead to adverse effects, for example, by altering the Maillard reaction products when LY038 corn is cooked.

The Maillard reaction ('browning reaction') is a non-enzymatic biochemical reaction that occurs during cooking of foods. In the presence of heat, amino acids and reducing sugars form complexes and result in odour and flavour molecules, most of which are poorly characterised and may include both beneficial and undesirable molecules. This reaction is responsible for the surface browning of cooked meats and bakery products.

However, Maillard reaction products produced during the processing or cooking of LY038 grain should not differ from those produced from conventional corn. With the exception of lysine, the amino acid profile of LY038 grain is comparable to conventional corn.

It is well accepted that any normally healthy compound, when consumed in sufficiently large quantities, may cause health problems, however the levels of lysine in LY038 corn do not warrant the level of concern displayed by the NZIGE. Lysine is an essential amino acid that cannot be produced by humans and therefore must be obtained from the diet. Corn is not traditionally a good source of lysine and even though LY038 contains significantly increased lysine, this is not significant in relation to other dietary sources of lysine. The levels of lysine in some commonly consumed foods are given in the following table.

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.

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.

R4: The Applicant should test the potential of *in planta*-produced cDHDPS to form amyloid fibrils and measure the cytotoxicity of aggregates and intermediate forms compared with native cDHDPS. It would be highly desirable to have the aggregation potential of cDHDPS correlated with changes in pH and other varying physical parameters of the chloroplast.

This concern has been raised by the NZIGE 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 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. 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 and do not believe that results of such a study as suggested in this recommendation would add to the overall body of information.

R5: In order to make an assessment of global changes in the transcriptome, and specific changes cause by the insertion(s) of I-DNA, the Authority should require micro-array descriptions capable of detecting novel RNA species in the modified plant, with the RNA source being the plant grown under a variety of relevant field conditions. The micro-array should comprehensively represent the genomes of the cultivar of maize modified and unmodified, and any novel RNA species should be tested against the human genome for RNAi activity.

The rationale behind this recommendation is presented in the NZIGE submission in Section 1.3. This section presents a summary of the biological properties of RNA that is generally accurate. However, the scientific evidence does not support the theory that RNA molecules in food can be transmitted to mammalian cells and exert effects on endogenous genes.

RNA is rapidly degraded even in intact cells. Following harvest, processing, cooking and digestion, it is unlikely that intact RNA would remain. Even if it did, it is very unlikely that it would enter human cells and be able to exert effects on endogenous genes.

What little is known about transcription levels of genes across entire plant genomes indicates that gene transcription may vary considerably even between closely related plants (Bruce *et al*., 2001; Guo *et al*., 2003; Umezawa *et al*., 2004). This high level of differential expression is thought to be due to a number of factors including environmental conditions and genotype. For this reason, analysis of changes in the transcriptome, while interesting, would not indicate whether these changes are within the range of natural variation nor would it provide any further information on the safety of the food.

R6: We recommend that I-DNA, especially the Glb1 promoter sequence, be analysed for putative mammalian transcription factor binding motifs.

This recommendation has been made due to concerns of the NZIGE that the intact Glb1 promoter sequence and the *cordapA* gene to which it is attached may be taken up by human cells following ingestion and cause the over-expression of cDHDPS in human cells or deregulate expression of endogenous genes.

The Glb1 promoter comes from corn and therefore has been consumed safely by humans for thousands of years. No safety concerns have been identified with the consumption of DNA from GM plants. A typical diet contains DNA from many sources – bacteria, plants, and animals. It is highly unlikely the Glb1 promoter from corn poses any greater risk than any other piece of DNA in the human diet. As only one copy of this promoter has been inserted into LY038, this will not appreciably increase the amount of this element ingested as it is estimated that the entire novel DNA insert in LY038 represents only 0.0002% of the corn genome.

R7: We recommend that a metabolomic analysis such as NMR combined with chemometrics and univariant statistics be supplied to the Authority by the Applicant.

The major nutrients and anti-nutrients present in corn have been identified by the OECD (OECD, 2002). These components were analysed in LY038 corn and non-transgenic control corn. In addition to those components identified by the OECD, specific lysine related metabolites were analysed and compared between conventional and LY038 corn grain.

In addition to targeted compositional analyses mentioned above, profiling methods (such as metabolomic analysis) may be able to provide insight into metabolic pathways in the plant (ILSI, 2004). However, as has been observed in relation to compositional analyses, the large range of natural variation that occurs between plants can mean that a statistically significant difference between the test and control plants for any given nutrient may not necessarily be biologically significant. This is also a major challenge with the use of profiling techniques and therefore these techniques need to be validated and the range of natural variation clearly established before they can be used for the purpose of safety assessment (ILSI, 2004).

Currently the internationally accepted practise for evaluation of new GM plants relies on a variety of data and information, including compositional analyses, to identify any unexpected changes in the plant, which are then subject to further scrutiny to determine their biological relevance and potential impact on food safety. FSANZ considers these data and information, when considered in total, provide assurance that the food is unlikely to have an adverse effect on human health.

R8: The Authority should require the Applicant, at a minimum, to supply data on the digestibility of the cDHDPS protein using a protocol consistent with the FAO/WHO standard (FAO/WHO, 2001) and the recommendations of Pusztai *et al.* (Pusztai, et al. 2003).

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.

R9: We recommend that a compositional analysis that includes the four commercial varieties used in MSL-18883 be requested by the Authority.

The reason the NZIGE has made this recommendation is elaborated on page 42 of the NZIGE submission. They are concerned by some apparent compositional differences (in amino acid levels) between the conventional corn lines used as controls in this study and the 99% tolerance interval from the conventional corn lines used as controls in study MSL-19172 (compositional analysis). Table 1 on page 42 of the NZIGE submission compares these values, however the data in the studies (MSL-18883 and MSL-19172) uses different units and therefore cannot be directly compared in this way.

Once the amino acid values in question are expressed using the same units (% total amino acids) they fall within the 99% tolerance interval as shown in the table below.

Component	99% tolerance interval from MSL-19172[1]	Average of four conventional corns from study MSL-18883[2,3]		LY038[3]	
Units	% total amino acids	mg/g	% total amino acids	mg/g	% total amino acids
Glutamic acid	16.76 – 22.36	**14.95**	19.21	**23.20**	20.14
Leucine	10.15 – 15.62	**9.28**	11.92	15.10	13.11
Methionine	1.54 – 2.41	1.63	2.09	**2.55**	2.21
Phenylalanine	4.49 – 5.68	**3.70**	4.75	**5.74**	4.98
Threonine	2.73 – 3.62	2.76	3.55	**3.87**	3.36

[1] Table 14, MSL-19172
[2] From Table 1, Appendix 1, MSL-18883
[3] Values in bold font from MSL-18883 are identified in the NZIGE submission as being outside the 99% tolerance interval from MSL-19172.

R10: We recommend that the Authority dismiss study MSL-18883 for purposes of assessing safety.

The study referred to in this recommendation is the broiler chicken feeding study. The NZIGE recommends dismissing this study due to confusion over the levels of amino acid in the control lines compared to those used in the compositional analyses. As discussed in Recommendation 9, these apparent compositional differences were due to differences in the units used.

Study MSL-18883 is a comparison of different corn diets, including LY038 corn and conventional corn (either supplemented with lysine or not), in supporting the growth and performance of broiler chickens. This study was submitted by the Applicant to demonstrate the wholesomeness of LY038 corn. The rapidly growing broiler is considered to be sensitive to changes in nutrient quality in diets, and therefore is often used as a model to assess normal growth and well-being and the wholesomeness of corn.

Normally, FSANZ does not require animal feeding studies to be submitted because when GM varieties have been shown to be compositionally equivalent to conventional varieties, feeding studies using target livestock species will add little to a safety assessment. In these circumstances the extent of the compositional data, molecular characterisation and toxicity / allergenicity data is considered sufficient to establish the nutritional adequacy and safety of the food. In cases where the composition of food has been significantly changed, as is the case with high-lysine corn, feeding studies with suitable livestock species may be useful to confirm the wholesomeness of the food.

It is important to note that comparative feedings studies, like the one submitted for high lysine corn, are not safety or toxicity studies and are only conducted with the purpose of demonstrating nutritional adequacy. Nevertheless, providing the study has been well conducted, the absence of adverse effects can provide additional assurances of safety. FSANZ has no valid reason to dismiss this particular study.

R11: The Authority should require the Applicant to submit data on cholesterol concentrations in serum and lipoprotein, and any changes in liver phospholipids, in animal feeding experiments.

As mentioned in the response to recommendation 10, FSANZ does not routinely require animal feeding studies to support the safety of a GM food. There are limitations in the extent to which such comparative feeding studies can be used to detect adverse effects because there are constraints on the amount of test material (in this case, high lysine corn) that can be incorporated into an animal's diet without creating nutritional imbalances. These studies are really only designed to demonstrate that the new GM food supports typical growth and wellbeing in the test animal. As a consequence, detailed blood analyses are rarely done.

These analyses have been requested by the NZIGE on the basis of a study in rabbits fed diets supplemented with various amino acids (Giroux *et al*., 1999). This study showed that diets high in lysine in combination with high levels of other amino acids led to increased serum cholesterol and phospholipids in the liver. Rabbits were fed diets containing 32.9 g lysine/kg diet, a very large amount of lysine compared to the levels found in LY038 corn (0.48% on a dry weight basis, or 4.8 g/kg dry weight). As the level of lysine in the supplemented diets given to the rabbits was very much higher than the levels found in high lysine corn, this study is not considered relevant to the safety of high lysine corn. In addition to this, corn is not a significant source of lysine in the human diet and even LY038 corn would not contribute significantly to lysine in the diet compared to other sources of dietary lysine.

There is no basis for the rationale that consumption of lysine from LY038 corn may have an effect on serum cholesterol.

R12: We recommend that the Authority request the Applicant to provide a valid sub-chronic toxicity study of a minimum of 6 months duration.

Long-term animal toxicity studies are not generally appropriate for the testing of whole foods. Such studies are commonly used in the safety assessment of discrete chemical compounds including pesticides, pharmaceuticals, industrial chemicals and food additives. In these cases, the test substance is well characterised, of known purity, of no nutritional value, and human exposure is generally low. It is therefore possible to feed such compounds to laboratory animals at a range of doses (using amounts greatly above expected human exposure levels) in order to identify any potential adverse effects. Establishing a dose-response relationship is a pivotal step in toxicological testing. By determining the level of exposure at which no adverse effects occur, a safe level of exposure for humans can be established which includes appropriate safety factors.

In contrast, traditional toxicological testing is not intended to be applied to the assessment of whole foods. Foods are complex mixtures of constituents and have wide variations in composition and nutritional value. Due to its bulk, a food can only be fed to laboratory animals at low multiples of the amounts that might be present in the human diet and it is therefore not possible to conduct normal dose-response experiments in the same way that these experiments are conducted for medicines and chemicals. In addition, a key factor in these experiments is the need to maintain the nutritional value and balance of the diet. A diet that consists entirely of a single food can cause adverse effects on nutritional status, potentially masking any other smaller effect of a component or components of the food being tested in the animals.

The observations from single food studies can therefore be confounded by a range of adverse effects not directly related to the food being tested.

Thus, a more focussed approached is required when the safety of a whole food is being considered. This approach is based on the principal that the safety of GM foods can be assessed by comparison to a conventional counterpart having a history of safe use, taking into account both intended and unintended effects. Rather than trying to identify every hazard associated with a particular food, the intention is to identify new or altered hazards relative to the conventional counterpart.

A component of this approach is to focus on the potential toxicity of any new proteins that have been introduced into the food through genetic modification. Because proteins are discrete chemical entities they can be fed to animals in large amounts, therefore it is possible to conduct animal toxicity studies to determine their safety. Acute toxicity testing has shown that the novel protein in LY038 corn, cDHDPS, is not toxic at high doses in rats.

However, a 3-month rat feeding study using LY038 corn was conducted by the Applicant and supplied to FSANZ. This study demonstrated no test substance adverse effects in rats fed up to 33% LY038 corn in their diets.

R13: We recommend that only after the Applicant demonstrates the safety of LY038 using a higher standard of *in vitro* and animal *in vivo* safety tests, then human tests should be completed before another application is lodged with the FSANZ.

The safety assessment for a GM commodity compares the molecular, toxicological and nutritional and compositional properties of the food to the non-GM form. The assessment focuses on the new gene product(s), including the intentional and unintentional effects of the genetic modification, and examines any compositional changes, including whether the genetic modification has altered the potential allergenicity and toxicity of the food. The assessment is therefore a comparative analysis using the commonly consumed conventional food as a benchmark for safety.

This comparative analysis is regarded by organisations such as the World Health Organisation (WHO)/Food and Agriculture Organisation (FAO), the Organisation for Economic Cooperation and Development (OECD) and the Codex Alimentarius Commission as the most practical approach for assessing the safety of a GM food. FSANZ regularly reviews procedures for assessment to ensure that recent scientific and regulatory developments are reflected in the process. At the international level, FSANZ is actively involved in the development of a framework for the assessment of GM foods within the Codex Alimentarius Commission.

Although the NZIGE does not specify the human tests that they would accept, human studies are not considered an appropriate or necessary part of the safety assessment process for GM foods.

R14: A plan for effective post-launch monitoring should be provided by the Applicant and the plan should be subject to a transparent review through the independent scientific community.

GM food products are not permitted on the market if any question associated with negative health effects is left unanswered during the pre-market safety assessment. For this reason post-market monitoring is not considered necessary or useful as there is no potential adverse health outcome to monitor.

Further, in Australia and New Zealand, as in most other countries, the responsibility for post-market surveillance is covered by an ongoing duty of care on the part of the developer. The developer is expected to monitor for existing and emerging risks that may be associated with its product and notify regulatory authorities whenever new information is uncovered.

R15: The Authority should clarify its proper jurisdiction with regard to this Application; in particular, it should clarify whether and how it is equipped to analyse the impact of the availability or non-availability of LY038 animal feed.

FSANZ is evaluating the safety for human consumption of food from LY038 corn. FSANZ does not have jurisdiction in relation to animal feed.

R16: We urge the Authority to disregard the declared intention of the Applicant to segregate LY038 from the human food supply, as it cannot bind the Applicant (or any other party) to this action once the Food Code has been amended. We recommend that the Authority ensure that this declared intention not be permitted to influence the rigour of the application or the analysis of its impacts.

FSANZ has conducted a comprehensive safety assessment on LY038 corn (Attachment 2 to the Draft Assessment Report), as it does on every new GM food and has not allowed the intention of the Applicant to segregate LY038 from the food supply to influence the rigour of the assessment.

R17: The Authority should clarify the reasoning behind its identification of Affected Parties; in particular, it should clarify why failing to amend the Food Code would prevent animal growers accessing LY038 feed.

A 'split approval' process with different standards for products destined for animal feed to those destined for the human food chain has led to problems internationally when products unapproved for human consumption inadvertently entered the food supply. To prevent this occurring, FSANZ and the Office of the Gene Technology Regulator (OGTR) have a Memorandum of Understanding (MoU) that no 'split approvals' will be made. As the OGTR is responsible for the assessment and regulation of GM feeds, the MoU sets out an agreement that where a GM food product such as corn has not undergone safety assessment by FSANZ, the OGTR would not approve its use as animal feed until such time that it is shown to be safe for human consumption, through assessment by FSANZ.

For this reason, FSANZ has agreed to assess the safety of this product for human consumption prior to it being used in animal feed. FSANZ has written the Regulatory Impact Statement to reflect this.

R18: The Authority or Applicant should provide evidence for its assertion that the cost to consumers of avoiding LY038, and to government of monitoring for the presence of LY038 in food, will be low.

FSANZ has an obligation to assess this GM food application and 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 objectives are also set out in Section 3 of the Draft Assessment Report on Application A549. Neither these three objectives nor the additional five points to which FSANZ must have regard specify that cost to government is a valid reason to reject an application. Furthermore, if FSANZ did not take this opportunity to assess high lysine corn for its safety for human consumption and it were later detected in the food supply, the cost to government may be significant.

FSANZ believes that the cost to consumers who wish to avoid GM products will not be significant even if this application were approved. Producers of high lysine corn will aim to sell their product at a premium price as animal feed due to the high levels of lysine. It is less likely that food industry would pay premium price for high lysine corn and therefore likely that the levels of high lysine corn entering the food supply would be small. Further, food containing high lysine corn is likely to be required to be labelled as GM and nutritionally different to conventional corn, allowing consumers to avoid this if they so chose.

R19: The Authority should not extend any approval of LY038 to any hybrid line derived from LY038. As in European regulation, 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.

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 approved. 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.

R20: The Authority should take into account the implications of approving this amendment of the Food Code for New Zealand's obligations under the Cartagena Protocol.

New Zealand has ratified the Cartagena Protocol on Biosafety, a multinational agreement to regulate the international trade of living modified organisms. The protocol is intended to help ensure that countries are themselves able to make decisions on import of living modified organisms to ensure their biodiversity is protected. Its main provisions relate to living modified organisms for intentional introduction into the environment (i.e. seeds for planting crops), although it has less extensive provisions relating to live modified organisms for food, feed and processing. If food derived from LY038 corn were to be approved by FSANZ, permission by other agencies in New Zealand (the Environmental Risk Management Authority) and Australia (the Office of the Gene Technology Regulator) would still be required before viable corn grain could be imported into either country.

It is anticipated that if LY038 grain enters the food supply in Australia and New Zealand, it will be via processed imported food products.

Information on the Cartagena Protocol is available on the New Zealand Ministry for the Environment's website at http://www.mfe.govt.nz/laws/meas/cartagena.html.

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