DRAFT RISK ANALYSIS REPORT

APPLICATION A387

Food derived from high oleic acid soybean lines G94-1, G94-19 and G168

Note:

This report is the "Full Assessment" as referred to in Section 15 of the *Australia New Zealand Food Authority Act (1991)*.

Public comments are now sought before completion of a Final Risk Analysis Report (referred to as the "Inquiry" in Section 16 of the Act). See under 'Invitation for Public Submissions' for details.

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Executive summary

Background

An application was received from Optimum Quality Grains LLC on 30 April 1999 for the approval of food from genetically modified (GM) soybean lines G94-1, G94-19 and G168. The soybeans have been genetically modified to contain high levels of oleic acid, a monounsaturated fatty acid. The GM soybeans are referred to as high oleic acid soybeans. This report describes the scientific assessment of the application.

Issues addressed during assessment

i. Safety Evaluation

The high oleic acid soybeans have been evaluated according to ANZFA's safety assessment guidelines. This involves an extensive analysis of the nature of the genetic modification together with a consideration of general safety issues, toxicological issues and nutritional issues associated with the new GM food. This approach can establish whether the foods produced from the high oleic acid soybeans are as safe and nutritious as foods produced from non-GM varieties of soybeans.

The detailed information available on the genetic modification to the soybeans shows that the novel genetic material is stably inserted and maintained over several generations and in different environments.

No new proteins are produced by any of the transferred genes however the high oleic acid soybeans were found to exhibit a slightly altered seed storage protein profile. The altered protein profile does not give rise to any significant increases in the allergen content of the high oleic acid soybeans and does not pose any other safety concerns.

Detailed compositional analyses of the high oleic acid soybeans demonstrated, as expected, that they are significantly changed with respect to their fatty acid profile. The most significant changes are to the oleic and linoleic acid content – the oleic acid content has been increased from 23.1% in the parental soybean to 83.8% in the high oleic acid soybeans and the linoleic acid content has been reduced from 55.4% to 2.2%. One minor, unexpected change did occur to the fatty acid profile of the soybeans resulting in the production of trace amounts of a fatty acid not normally found in non-GM soybeans. This fatty acid, however, is commonly found in hydrogenated soybean oil. The overall change to the fatty acid profile of the soybeans does not pose any safety concerns. In all other respects, the high oleic acid soybeans are compositionally equivalent to non-GM varieties of soybean.

The impact on human health from potential transfer of novel genetic material to cells in the human digestive tract has also been considered. The presence of novel genetic material, including an ampicillin resistance gene, in the high oleic acid soybeans is not considered to pose any additional safety concerns.

In assessing all of the above data, ANZFA has concluded that the high oleic acid soybeans do not raise any public health and safety concerns.

ii. Labelling

On the basis of the data considered in the safety evaluation, the high oleic acid soybeans are significantly changed with respect to their fatty acid profile. Therefore, where food from the high oleic acid soybeans contains new or altered genetic material, labelling will be required to indicate the origin and nature of the characteristics that have been modified. Food derived from the high oleic acid soybeans that does not contain new or altered genetic material will still have to be labelled according to the general labelling provisions as they relate to the naming of food. These provisions require that the true nature of the food be reflected on the label of the product in question. Therefore refined oil derived from the high oleic acid soybeans - which would not be expected to contain any new or altered genetic material - will still need to be labelled to distinguish it from standard soybean oil.

It should be noted that the labelling provisions in Standard A18 are in the process of being amended. This may result in changes to the way in which some GM foods, including those derived from high oleic acid soybeans, are labelled.

iii. Public Submissions

Forty-four public submissions were received in response to notification of this application, of which only four were supportive. Those opposing the application did so primarily on the basis that they perceive GM food to be unsafe. The food safety concerns raised in submissions have been addressed by the safety assessment report.

Conclusion

ANZFA considers that food derived from high oleic acid soybeans is as safe for human consumption as food from other commercial soybean varieties and is therefore proposing an amendment to the Australian *Food Standards Code* to give approval to such food. Based on the data submitted in the present application, ANZFA proposes that high oleic acid soybeans are significantly changed compared to non-GM soybeans with respect to their fatty acid profile, and that mandatory labelling will be required.

ANZFA will now seek public comment on the proposed amendment to Standard A18 of the *Food Standards Code* (in accordance with the procedures described in section 17 of the *Australia New Zealand Food Authority Act 1991*).

INVITATION FOR PUBLIC SUBMISSIONS

The Authority has completed a Draft Risk Analysis Report on this application, (referred to as the 'Full Assessment' in section 15 of the Act), which includes a draft Safety Assessment report and a draft variation to the Australian *Food Standards Code.* The Authority now seeks public comment on the draft Safety Assessment Report, the draft variation to the *Food Standard Code,* and the Regulatory Impact Assessment before preparing a Final Risk Analysis Report (referred to as the 'Inquiry' in section 16 of the Act).

Written submissions containing technical or other relevant information, which will assist the Authority in preparing the Final Risk Analysis Report for this application, are invited from interested individuals and organisations. Technical information presented should be in sufficient detail to allow independent scientific assessment.

Submissions providing more general comment and opinion are also invited. The Authority's policy on the management of submissions is available from the Standards Liaison Officer upon request.

The processes of the Authority are open to public scrutiny, and any submissions received will ordinarily be placed on the public register of the Authority and made available for inspection. If you wish any information contained in a submission to remain confidential to the Authority, you should clearly identify the sensitive information and provide justification for treating it as commercial-in-confidence. The *Australia New Zealand Food Authority Act 1991* requires the Authority 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.

All correspondence and submissions on this matter should be addressed to the **Project Manager - Application A387** at one of the following addresses:

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Submissions should be received by the Authority by 30 August 2000.

General queries on this matter and other Authority business can be directed to the Standards Liaison Officer at the above address or by Email on slo@anzfa.gov.au. Submissions should not be sent by Email as the Authority cannot guarantee receipt. Requests for more general information on the Authority can be directed to the Information Officer at the above addresses.

Introduction

The Australia New Zealand Food Authority (ANZFA) is a bi-national statutory body responsible for making recommendations on food standards which, when approved by the Australia New Zealand Food Standards Council (ANZFSC), are adopted by reference and without amendment into food law. ANZFA is currently working to establish a joint *Australia New Zealand Food Standards Code* that will apply in both countries. In the interim, a system of dual standards operates for the majority of the food standards. Standard A18 – Food Produced using Gene Technology has been accepted by New Zealand, and currently applies in both countries.

Standard A18 was adopted by ANZFSC as a joint Australia/New Zealand standard in July 1998 and came into force on 13 May 1999. Under this Standard, the sale of food produced using gene technology is prohibited unless the food is included in the Table to Clause 2 of the Standard. The Standard requires that a pre-market safety assessment be conducted on all foods produced using gene technology. However, the Standard provides interim arrangements for those foods currently on the market provided that an application was accepted by ANZFA on or before 30 April 1999, that the food is lawfully permitted in a country other than Australia or New Zealand, and that ANZFSC has not become aware of evidence that the food poses a significant risk to public health and safety.

Background to the application

ANZFA received an application from Optimum Quality Grains on 30 April 1999 to amend the Australian *Food Standards Code* to include food derived from high oleic acid soybean lines G94-1, G94-19 and G168 in the Table to Clause 2 of Standard A18 – Food Produced using Gene Technology.

The three lines of soybeans have been genetically modified to contain high levels of oleic acid, a monounsaturated fatty acid. The GM soybeans are referred to as high oleic acid soybeans.

The high oleic acid soybeans were generated by the suppression of a soybean gene involved in the metabolic pathway responsible for fatty acid biosynthesis.

High oleic acid soybeans are not currently grown in either New Zealand or Australia. The principle food product derived from the high oleic acid soybeans will be the oil. It is anticipated that the high oleic acid soybean oil will be used in spraying (e.g., of crackers and breakfast cereals) and frying applications in the food industry and food services and might replace heat stable fats and oils, such as hydrogenated soybean oil or palm olein/vegetable oil blends. A consequent by-product of the soybean processing will be soybean meal and soy isolates. These products could appear in a wide range of processed foods such as soymilks and processed meats.

Soybean oil has poor oxidative stability due to naturally high levels of polyunsaturated fatty acids (such as linoleic acid). High oleic acid soybean oil is considered to have superior properties to that of standard soybean oil because of its reduced levels of the oxidatively unstable polyunsaturated fatty acids. This means that high oleic acid soybean oil may be used for a number of food applications, including deep fat frying, without the need for additional

processing, such as chemical hydrogenation. High oleic acid soybean oil is also considered to offer improved nutritional properties compared to conventional soybean oil or partially hydrogenated soybean oil because of the increased levels of monounsaturated fatty acids and because its replacement of other oils may lead to a decreased consumption of saturated fatty acids.

Public consultation

ANZFA completed a Notice of Application (formally referred to as the Preliminary Assessment Report) upon receipt of the application and called for public comment on 3 November 1999. A total of 44 submissions were subsequently received. Attachment 5 contains a summary of the submissions.

During the ANZFA assessment process, comments are also sought internationally from other Members of the World Trade Organization (WTO). As Members of the WTO, Australia and New Zealand are signatories to the agreements on the Application of Sanitary and Phytosanitary Measures (SPS Agreement) and on Technological Barriers to Trade (TBT Agreements) (for further details on WTO, see Attachment 4). In some circumstances, Australia and New Zealand have an obligation to notify the WTO of changes to food standards to enable other member countries of the WTO to make comment.

In the case of foods produced using gene technology, changes to Standard A18 have been notified to the WTO because there is significant international interest in the safety of these foods.

Issues addressed during assessment

1. Safety assessment

The safety assessment was performed according to the safety assessment guidelines prepared by ANZFA[1] and considered the following issues: (1) the nature of the genetic modification; (2) general safety issues such as novel protein expression and the potential for transfer of novel genetic material to cells in the human digestive tract; (3) toxicological issues; and (4) nutritional issues.

Nature of the genetic modification

Three genes were transferred to the high oleic acid soybeans using the method of particle bombardment — a second copy of the soybean *GmFad 2-1* gene, and the bacterial *uidA* and *bla* genes.

The *GmFad 2-1* gene encodes the fatty acid desaturase responsible for catalysing the synthesis of linoleic acid from oleic acid. Linoleic acid is one of the major polyunsaturated fatty acid constituents of soybean oil and is normally present at >55% of the total fatty acids. The transfer of a second copy of the fatty acid desaturase gene to the soybean plant results in a phenomenon known as "gene silencing" which causes both the original and the transferred fatty acid desaturase gene to be "switched off". This blocks the step in the metabolic pathway

[1] ANZFA (1999) Guidelines for the safety assessment of foods to be included in Standard A18 – food produced using gene technology.

responsible for the synthesis of linoleic acid and leads to the accumulation of oleic acid as well as the concomitant decrease of linoleic acid, in the developing soybean seed.

The two other genes were used as markers to assist in the selection of transformed cells (i.e., cells to which the *GmFad 2-1* gene had been transferred). The *uidA* gene encodes the enzyme β-glucuronidase and acts as a colourimetric marker. The *bla* gene encodes the enzyme β-lactamase and confers resistance to some β-lactam antibiotics, such as penicillin and ampicillin.

All three genes are present in all high oleic acid soybean lines and appear to be stably integrated and maintained in the soybean plants over multiple generations and in various environments.

General safety issues

Soybeans are grown as a commercial crop in over 35 countries worldwide and have a long history of safe use as human food. The main food product to be derived from the high oleic acid soybeans will be the oil. High oleic soybean oil is expected to replace fats and oils such as hydrogenated soybean and rapeseed oil or palm olein/vegetable oil blends.

Extensive analyses of the high oleic acid soybeans have demonstrated that none of the transferred genes give rise to a protein product, meaning that no new proteins are expressed in any of the high oleic acid soybean lines.

The impact on human health from potential transfer of novel genetic material from high oleic acid soybeans to cells in the human digestive tract was evaluated. It was concluded that the probability of transfer was extremely low but that, in the case of the ampicillin resistance gene, should it occur the health impacts would be negligible because ampicillin resistant bacteria are already commonly found in the human digestive tract and in the environment.

Toxicological issues

Although no new proteins are expressed in any of the high oleic acid soybean lines, they do exhibit a slightly altered seed storage protein profile. Allergenicity testing confirmed, however, that the alteration to the protein profile has not given rise to any significant increase to the allergenicity of the high oleic acid soybeans, when compared to non-GM soybeans.

The only naturally-occurring toxins in soybeans are lectins. The high oleic acid soybean lines exhibit slightly elevated lectin levels when compared to the control soybean line but these levels are well within the literature reported range for soybeans. As lectins are readily degraded upon heating and there are no human food uses for raw soybeans, the slightly elevated levels observed are not a cause for concern.

Nutritional issues

Detailed compositional analyses were done to establish the nutritional adequacy of the high oleic acid soybeans. Constituents analysed were proximate (crude fat/protein, fibre, ash), amino acid, fatty acid, vitamin and mineral, isoflavone, and anti-nutrient content.

These analyses confirmed that the high oleic acid soybeans are significantly changed with respect to their fatty acid profile. The major change has been to the oleic acid content has been increased from a mean of 23.1% in the parental soybean to 83.8% in the high oleic soybean lines. The linoleic acid content has been concomitantly decreased from a mean level of 55.4% to a mean level of 2.2%. Small reductions in the levels of palmitic and linolenic acid were also observed. High oleic acid levels are commonly consumed in other premium edible oils therefore the consumption of high levels of oleic acid does not raise any safety concerns.

The compositional analyses also revealed the unexpected occurrence of trace amounts (less than 1%) of an isomer of linoleic acid in the high oleic acid soybeans. This isomer is not present in the parental soybean line but is normally found in commonly consumed foods such as hydrogenated soybean oils and butterfat. It is present at levels in the high oleic acid soybeans which are comparable to those levels found in hydrogenated soybean oils and butterfat, therefore, its presence is not considered to pose any toxicological or nutritional concerns.

In all other respects, the high oleic acid soybeans are compositionally equivalent to the parental soybean line and other commercial varieties of soybean.

Although not essential for establishing the safety of the food, animal feeding studies with the high oleic acid soybeans were evaluated as additional supporting data. These studies demonstrated that the high oleic acid soybeans are equivalent to non-GM soybeans in their ability to support typical growth and well-being, thus confirming the nutritional adequacy of the high oleic acid soybeans.

A study was also undertaken to assess the human nutritional impact of the use of high oleic acid soybean oil as a replacement for frying fats. The study, modelled on British diets, concluded that the use of high oleic soybean oil might lower dietary linoleic acid intake somewhat (by an absolute maximum of 29%), but it would not do so to any level that would be a public health concern in terms of cardiovascular disease. This level of reduction is likely to be smaller in Australian diets because of the lower contribution to energy intake from polyunsaturated fatty acids. Overall, the conclusion of the study was that the nutritional impact of the use of high oleic acid soybean oil was likely to be beneficial because diets incorporating high oleic acid soybean oil show decreased saturated fatty acid intakes and this is likely to reduce risk factors for cardiovascular disease.

Conclusion

Based on the data submitted in the present application, the high oleic acid soybeans are significantly changed with respect to their fatty acid profile but are equivalent to non-GM soybeans in terms of their safety and nutritional adequacy.

2. Labelling of food derived from high oleic acid soybeans

Clause 3 of Standard A18 prescribes mandatory labelling of a food produced using gene technology when it contains new or altered genetic material and where it is not substantially equivalent in any characteristic or property of the food. As the high oleic acid soybeans have been significantly changed with respect to their fatty acid profile, any food derived from them that contains new or altered genetic material will require mandatory labelling. Food derived

from the high oleic acid soybeans that does not contain new or altered genetic material will still need to comply with the general labelling provisions of food law which require that the true nature of the food be reflected on the label of the product in question.

It should be noted that the proposed amendments to the labelling provisions of Standard A18 could result in some changes to the way in which food derived from high oleic acid soybeans is labelled in the future.

3. Issues arising from public submissions

3.1 General issues

Of the 44 submissions received, only a small number addressed issues specific to this application. Rather, the majority of submissions raised issues of a general nature relating to gene technology or issues that had already been addressed in the safety assessment report (see Attachment 2). A discussion of some of the general issues in relation to gene technology that were raised in public submissions can be found in Attachment 6.

3.2 Specific issues

This section of the report will only address those issues raised in public submissions that are specific to an assessment of this application.

(i) Phytoestrogens

The Consumers' Association of South Australia and the National Council of Women of Australia submitted that new research has shown that GM soybeans may be a less potent source of phytoestrogens than conventional soybeans.

Response

The isoflavone content of non-GM soybeans (which includes phytoestrogens) has been found to vary widely. For example, in a study done in the United States the content of total daidzein in soybeans was reported to vary from 295 – 1527 μg/g dry weight and, for total genistein, from 416 – 2676 μg/g dry weight.

Data on the isoflavone content of the high oleic acid soybean lines was provided with the application and was evaluated in the safety assessment (Attachment 2). The data shows that the high oleic acid soybeans do not contain significantly less isoflavones compared to the parental soybean line, or other commercial varieties of soybeans.

(ii) Nutritional impact of the high oleic acid soybeans

The Consumers' Association of South Australia and the National Council of Women of Australia submitted that raising the amount of a nutrient in a food may have unknown drawbacks such as affecting the efficacy of other nutrients.

Response

High oleic acid soybean oil contains a mean level of 83.8% oleic acid and 2.2% linoleic acid. This compares to mean levels of 23.1% oleic acid and 55.4% linoleic acid in conventional soybean oil. The fatty acid content of high oleic acid soybean oil is therefore more comparable to other commonly consumed premium edible oils, such as olive oil and high oleic sunflower and canola oils. Humans therefore already have a history of consuming oils with similar fatty acid profiles to that of high oleic acid soybean oil.

To more thoroughly assess the nutritional impact of high oleic acid soybean oil the applicant commissioned a report from Nutriscan Ltd[2] on the effect of high oleic acid soybean oil on the balance of dietary fats in the human diet. The study used the database of the Dietary and Nutritional Survey of British Adults (aged 16-64 years) and a report of the study was submitted with the application.

The study found that, if high oleic acid soybean oil were to replace all oils present in savoury snacks, fried potatoes including chips and vegetables and was assumed to account for 17% of the fat in all fried meat, eggs and fish, there would be a probable reduction of 5% in the intake of saturated fatty acids, a 19% rise in monounsaturated fatty acid intake and a 29% decrease in the polyunsaturated fatty acid intake. This represents a worst-case scenario. The impact was found to very similar to if olive oil were to replace all of the fats considered in the analysis.

The study concluded although the use of high oleic soybean oil might lower dietary linoleic acid intake somewhat (by an absolute maximum of 29%), it would not do so to any level that would be a public health concern in terms of cardiovascular disease. Overall, the conclusion of the study was that the nutritional impact of the use of high oleic acid soybean oil was likely to be beneficial because diets incorporating high oleic acid soybean oil show decreased saturated fatty acid intakes and this is likely to reduce risk factors for cardiovascular disease.

4. Risk management

Under Standard A18 a GM food must undergo a safety assessment in accordance with ANZFA's safety assessment guidelines. The requirement for the food to be labelled must also be assessed in accordance with the labelling criteria specified in Clause 3 of the standard.

On the basis the conclusions of the safety assessment report, together with a consideration of the public submissions, it is proposed that Table 1 to Clause 2 of Standard A18 be amended to include food from high oleic acid soybean lines G94-1, G94-19 and G168. The proposed amendment is provided in Attachment 1.

In relation to labelling of the food, the safety assessment report found that high oleic acid soybean lines G94-1, G94-19 and G168 are significantly changed with respect to their fatty acid profile when compared to non-GM soybeans. Therefore, under the current standard, mandatory labelling is required.

[2] A non-profit Campus Company of Trinity College, Dublin, Ireland.

In relation to the concerns raised in the public submissions with regard to gene technology and GM food, ANZFA is currently preparing a public discussion paper on the safety assessment process for GM food[3]. This will be widely available and may assist in addressing some of the concerns raised by the public. Other government and industry bodies are also addressing the broader concerns in relation to gene technology.

Regulatory impact assessment

The benefits and costs associated with the proposed amendment to Standard A18 have been analysed in a draft Regulatory Impact Assessment (Attachment 3). The benefits of the proposed Standard A18 amendment to approve food from high oleic acid soybeans primarily accrue to the food industry and government, with potentially a small benefit to the consumer.

Conclusions

It is concluded that:

- the introduced genes in high oleic acid soybean lines G94-1, G94-19 and G168 are not considered to produce any increased risk to public health and safety;
- high oleic acid soybean lines G94-1, G94-19 and G168 are significantly changed with respect to their fatty acid profile but are equivalent to non-GM soybeans in terms of their safety and nutritional adequacy;
- food derived from high oleic acid soybean lines G94-1, G94-19 and G168 will require labelling if it contains new or altered genetic material. Food derived from high oleic acid soybean lines G94-1, G94-19 and G168 which does not contain new or altered genetic material is not currently required to be labelled under Standard A18. Such food will still have to be labelled in a way that describes the true nature of the food. The proposed amendments to the labelling provisions of Standard A18 could result in some changes to the way in which food derived from high oleic acid soybeans is labelled in the future; and
- the benefits to government, consumers and industry associated with the proposed amendment outweigh the costs.

ATTACHMENTS

1. Draft variation to the Australian *Food Standards Code*
2. Draft safety assessment report
3. Draft regulatory impact assessment
4. World Trade Organisation Agreements
5. Summary of public comments
6. General issues raised in public comments

[3] ANZFA (2000) GM foods and the consumer: ANZFA's safety assessment process for genetically modified foods. ANZFA Occasional Paper Series No. 1.

ATTACHMENT 1

DRAFT VARIATION TO THE AUSTRALIAN FOOD STANDARDS CODE

A387 - FOODS FROM HIGH OLEIC ACID SOYBEANS

Standard A18 *is varied by inserting into Column 1 of the Table to clause 2 -*

Food derived from high oleic acid soybean lines G94-1, G94-19 and G168.

ATTACHMENT 2

DRAFT

SAFETY ASSESSMENT REPORT

A387 – Food derived from high oleic acid soybean lines G94-1, G94-19 and G168

SUMMARY AND CONCLUSIONS

Nature of the genetic modification

Three lines of a new variety of soybean (G94-1, G94-19 and G168), high in the monounsaturated fatty acid oleic acid, were generated by the transfer of a second copy of a soybean fatty acid desaturase gene (*GmFad 2-1*) to a high yielding commercial variety of soybean (line A2396). The fatty acid desaturase is responsible for the synthesis of linoleic acid, which is the major polyunsaturated fatty acid present in soybean oil. The presence of a second copy of the fatty acid desaturase gene causes a phenomenon known as "gene silencing" which results in both copies of the fatty acid desaturase gene being "switched off", thus preventing linoleic acid from being synthesised and leading to the accumulation of oleic acid in the developing soybean seed.

Other genes transferred along with the *GmFad 2-1* gene were the *uidA* gene and the *bla* gene. The *uidA* gene is a colourimetric marker used for selection of transformed plant lines during the soybean transformation procedure. It codes for the enzyme β-glucuronidase and is derived from the bacterium *Escherichia coli*. The *bla* gene is a marker used to select transformed bacteria from non-transformed bacteria during the DNA cloning and recombination steps undertaken in the laboratory prior to transformation of the plant cells. It codes for the enzyme β-lactamase and confers resistance to some β-lactam antibiotics, such as penicillin and ampicillin.

The transferred genes were all found to be stably integrated into the genome of the high oleic acid soybean lines and are all phenotypically and genetically stable over multiple generations and in various environments.

General safety issues

Soybeans are grown as a commercial crop in over 35 countries worldwide and have a long history of safe use as human food. The major food product to be derived from the high oleic acid soybeans will be the oil. High oleic acid soybean oil will be predominantly used in spraying and frying applications and might replace heat stable fats and oils such as hydrogenated soybean and rapeseed oil or palm olein/vegetable oil blends.

Extensive analyses of the high oleic acid soybeans have demonstrated that none of the transferred genes give rise to a protein product, meaning no new proteins are expressed in any of the high oleic acid soybean lines.

One of the important issues to consider in relation to genetically modified foods is the impact on human health from potential transfer of novel genetic material to cells in the human digestive tract. Much of the concern in this regard is with the presence of antibiotic resistance genes in genetically modified foods. In the case of the high oleic acid soybeans, it was concluded that the *bla* gene would be extremely unlikely to transfer to bacteria in the human digestive tract because of the number and complexity of the steps that would need to take place consecutively. More importantly, however, in the highly unlikely event that transfer did occur, the human health impacts would be negligible because ampicillin resistant bacteria are already commonly found in the human gut and in the environment. Transfer of other novel genetic material from the high oleic acid soybeans to human cells via the digestive tract was also considered to be equally unlikely. As the amount of novel genetic material in the

high oleic acid soybeans is minute compared to the total amount of DNA present it is unlikely to pose any special additional risks compared with the large amount of DNA naturally present in all foods.

Toxicological issues

Although no new proteins are expressed in any of the high oleic acid soybean lines, they were found to exhibit a slightly altered seed storage protein profile. Allergenicity testing confirmed, however, that the altered protein profile does not give rise to any significant differences between the allergen content of the high oleic acid soybeans and the parental soybean line A2396. Nor did the altered protein profile lead to significant changes to the total protein content of the high oleic acid soybeans.

The only naturally-occurring toxins in soybeans are lectins. The high oleic acid soybean lines exhibit slightly elevated lectin levels when compared to the control but these levels are well within the literature reported range for soybeans. As lectins are readily degraded upon heating and there are no human food uses for raw soybeans, the slightly elevated levels observed are not a cause for concern.

Nutritional issues

Detailed compositional analyses were done to establish the nutritional adequacy of the high oleic acid soybeans. Analyses were done of proximate (crude fat/protein, fibre, ash), amino acid, fatty acid, vitamin and mineral, isoflavone, and anti-nutrient content. These analyses confirmed that the high oleic acid soybeans are significantly changed with respect to their fatty acid profile. The mean oleic acid content has been increased from 23.1% in the parental soybean to 83.8% in the high oleic soybean lines and the linoleic acid content has been concomitantly decreased from a mean level of 55.4% to a mean level of 2.2%. Small reductions in the levels of palmitic and linolenic acid were also observed. High oleic acid levels are found in other commonly consumed premium edible oils (e.g., olive oil and high oleic sunflower and canola oil). The consumption of high levels of oleic acid is not considered to pose any safety concerns.

The compositional analyses revealed the unexpected occurrence of trace amounts (less than 1%) of an isomer of linoleic acid in the high oleic acid soybeans. This isomer is not present in the parental soybean line but is normally found in commonly consumed foods such as hydrogenated soybean oils and butterfat. It is present at levels in the high oleic acid soybeans that are comparable to the levels found in hydrogenated soybean oils and butterfat. Its presence is not considered to pose any toxicological or nutritional concerns.

Based on the data submitted in the application, in all other respects, the high oleic acid soybeans are compositionally equivalent to the parental soybean line and other commercial varieties of soybean. In addition, the results of two animal feeding studies, with pigs and chickens, confirm that the high oleic acid soybeans are also equivalent to other commercial varieties of soybean with respect to its ability to support typical growth and well-being.

A study was also undertaken to assess the human nutritional impact of the use of high oleic acid soybean oil as a replacement for frying fats. The study concluded that the use of high oleic soybean oil might lower dietary linoleic acid intake somewhat (by an absolute maximum of 29%), but it would not do so to any level that would be a public health concern in terms of

cardiovascular disease. Overall, the conclusion of the study was that the nutritional impact of the use of high oleic acid soybean oil was likely to be beneficial because diets incorporating high oleic acid soybean oil show decreased saturated fatty acid intakes and this is likely to reduce risk factors for cardiovascular disease.

Conclusion

Based on the data submitted in the present application, the high oleic acid soybeans are significantly changed with respect to their fatty acid profile but are equivalent to non-GM soybeans in terms of their safety and nutritional adequacy.

1. BACKGROUND

Optimum Quality Grains LLC (a joint venture between DuPont and Pioneer Hi-Bred International, Inc) have made an application to ANZFA to amend Standard A18 of the Australian *Food Standards Code* to include food derived from soybeans that have been genetically modified to contain increased levels of oleic acid, a monounsaturated fatty acid. The soybeans are referred to as *high oleic acid soybeans*.

The high oleic acid trait was generated by the transfer of a second copy of a soybean fatty acid desaturase gene (*GmFad 2-1*) to a high yielding commercial variety of soybean. The fatty acid desaturase is responsible for the synthesis of linoleic acid, which is the major polyunsaturated fatty acid present in soybean oil. The presence of a second copy of the fatty acid desaturase gene causes a phenomenon known as "gene silencing" which results in both copies of the fatty acid desaturase gene being "switched off", thus preventing linoleic acid from being synthesised and leading to the accumulation of oleic acid in the developing soybean seed.

Soybean oil has poor oxidative stability due to naturally high levels of polyunsaturated fatty acids (such as linoleic acid). High oleic acid soybean oil is considered to have superior properties to that of standard soybean oil because of its reduced levels of the oxidatively unstable polyunsaturated fatty acids. This means that high oleic acid soybean oil may be used for a number of food applications, including deep fat frying, without the need for additional processing, such as chemical hydrogenation. High oleic acid soybean oil is also considered to offer improved nutritional properties compared to conventional soybean oil or partially hydrogenated soybean oil because of the increased levels of monounsaturated fatty acids.

2. DESCRIPTION OF THE MODIFICATION

2.1 Methods used in the genetic modification

Elite soybean (*Glycine max*.) line A2396 was co-transformed with plasmids pBS43 and pML102. Both plasmids were introduced into soybean meristem tissue as purified plasmid DNA via the method of particle bombardment.

2.2 Function and regulation of the novel genes

Co-transformation of soybean with the plasmids pBS43 and pML102 resulted in the transfer of three gene expression cassettes - *GmFad 2-1*, *uidA* (otherwise known as GUS), and *dapA*. These expression cassettes are described in Table 1 below.

Table 1: Description of the *GmFad 2-1*, *uidA* and *dapA* gene expression cassettes in pBS43 and pML102

Cassette	Genetic element	Source	Function
***GmFad 2-1* expression cassette (pBS43 only)**	β-conglycinin promoter	α^1-subunit of β-conglycinin seed storage protein of soybean.	Seed specific promoter that allows high level gene expression during seed development.
	GmFad 2-1 coding region	Protein coding sequence of the δ-12 fatty acid desaturase from soybean.	The endogenous enzyme adds a second double bond to oleic acid thus converting it to linoleic acid.
	phaseolin 3' terminator	The 3' terminator region from the phaseolin seed storage protein of green bean *Phaseolis vulgaris.*	Contains signals for termination of transcription and directs polyadenylation.
GUS expression cassette (pBS43 only)	35S promoter	A promoter derived from the cauliflower mosaic virus (CaMV).	Promoter of high level constitutive gene expression in plant tissues.
	Cab 22L non-translated leader	The 5' untranslated leader from the photosynthetic *22L* chlorophyll a/b binding protein (*Cab22L*) promoter of *Petunia hybrida* var. Mitchell.	The untranslated leader sequence helps to stabilise mRNA and improve translation.
	uidA coding region	Protein coding sequence of the enzyme β-glucuronidase (*uidA* gene) from *Escherichia coli* (Jefferson *et al* 1985).	Colourimetric marker used for selection of transformed plant lines.
	NOS 3'	The 3' terminator region of the nopaline synthase gene from the Ti plasmid of *Agrobacterium tumefaciens.*	Contains signals for termination of transcription and directs polyadenylation.
***dapA* expression cassette (pML102 only)**	Kti3 promoter	Promoter from Kunitz trypsin inhibitor gene 3 of soybean.	Seed specific promoter that allows high level gene expression during seed development.
	ssu CTC	The N-terminal chloroplast transit peptide sequence from the soybean small subunit of Rubisco.	Directs the protein into the chloroplast which is the site of lysine biosynthesis.
	dapA coding region	Coding sequence of the *Corynebacterium dapA* gene encoding the lysine insensitive version of the enzyme dihydrodipicolinic acid synthase (DHDPS).	Expression of *Corynebacterium* DHDPS deregulates the lysine biosynthetic pathway resulting in accumulation of free lysine (Falco *et al* 1995).
	Kti3 3' terminator	The 3' terminator region from Kunitz trypsin inhibitor gene 3 from soybean.	Contains signals for termination of transcription and directs polyadenylation.

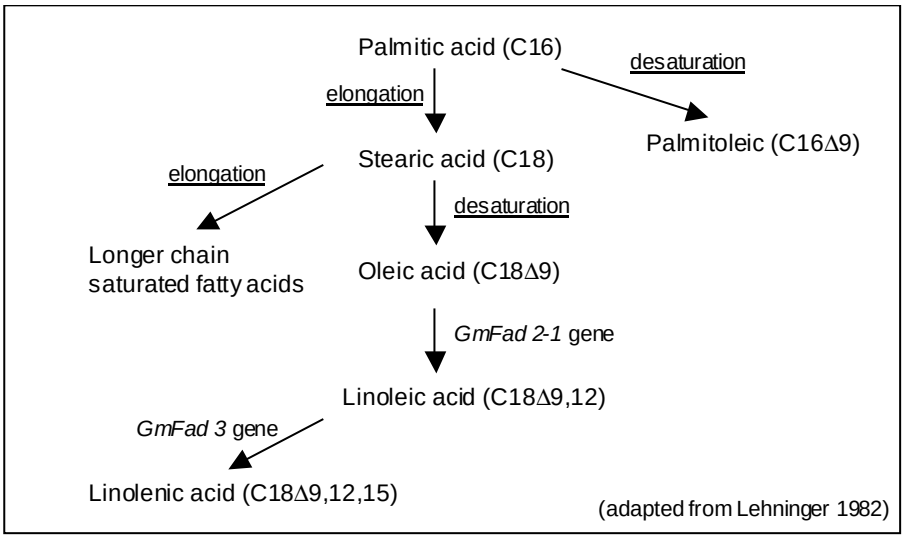
The *GmFad 2-1* gene

The synthesis of polyunsaturated fatty acids in developing oilseeds is catalysed by two membrane-associated desaturases that sequentially add a second and third double bond to oleic acid (Kinney 1994). The second double bond, converting oleic acid to linoleic acid, is added at the δ-12 (n-6) position by a δ-12 desaturase, encoded by the *GmFad 2-1* gene

(Okuley *et al* 1994, Heppard *et al* 1996). The third double bond, converting linoleic acid to linolenic acid, is added at the n-3 (δ-15) position by an n-3 desaturase, encoded by the *GmFad 3* gene (Yadav *et al* 1993). The *GmFad 2-1* gene used to genetically modify the soybeans is itself derived from soybean.

In soybean, there are two Fad 2 genes, but only the *GmFad 2-1* gene is expressed in the developing seed (Heppard *et al* 1996). The expression of *GmFad 2-1* increases during the period of oil deposition, starting around 19 days after flowering, and its gene product is responsible for the synthesis of the polyunsaturated fatty acids found in the oil fraction. The second Fad 2 gene (*GmFad 2-2*) is expressed in the seed, leaf, root and stem at a constant level and its gene product is responsible for the synthesis of the polyunsaturated fatty acids present in cell membranes.

The pathway for the synthesis of long chain fatty acids in plants is depicted below.



The presence of a second copy of the *GmFad 2-1* gene in the soybean causes a phenomenon known as "gene silencing" which results in both copies of the *GmFad 2-1* gene (the transferred copy as well as the original soybean copy) being "switched off", thus preventing linoleic acid from being synthesised and leading to the accumulation of oleic acid in the developing soybean seed.

Gene silencing, also known as co-suppression, is a phenomenon that is sometimes observed when plants are genetically modified to contain new or additional copies of genes and is a means by which plant genes can be deliberately "switched off" so that they no longer give rise to a protein product in the cell (US patent 5034323). Gene silencing is thought to occur by one of two mechanisms (Reviewed in Matzke and Matzke 1995, Finnegan and McElroy 1996, Stam *et al* 1997). In one, inactivation occurs by repression of RNA transcription and is associated with methylation of the promoter. The second results in the failure to accumulate messenger RNA in the cytoplasm, probably due to targeted degradation of mRNA.

The *dapA* gene

The *dapA* gene codes for the enzyme dihydrodipicolinic acid synthase (DHDPS), which is responsible for catalysing the first step in the metabolic pathway for the synthesis of the essential amino acid lysine (Brock *et al* 1984). This reaction is the condensation of aspartate

semi-aldehyde with pyruvate to form 2,3-dihydrodipicolinate. The reaction takes place in the chloroplast of higher plants as well as in many bacteria. Animals are incapable of synthesising lysine; therefore they must obtain their lysine through dietary sources. In plants, DHDPS is inhibited by lysine and is the major regulatory enzyme of lysine biosynthesis. The *dapA* gene transferred to the soybeans codes for a form of DHDPS that is insensitive to inhibition by lysine. The gene was derived from *Corynebacterium*.

In previous experiments it has been shown that expression of the lysine-insensitive DHDPS, encoded by the *Corynebacterium dapA* gene, will result in more than a 100-fold increase in the accumulation of free lysine in the seeds, essentially doubling total seed lysine content (Falco *et al* 1995).

The objective of transforming soybean with both the soybean *GmFad 2-1* gene and the *Corynebacterium dapA* gene was to produce transgenic soybeans with increased lysine in their meal fraction, due to expression of the lysine insensitive form of DHDPS, and a reduced level of polyunsaturated fatty acids in their oil fraction, due to silencing of the *GmFad 2-1* gene (described above).

Other genetic elements

In addition to the gene expression cassettes described above, a number of other genetic elements, including an antibiotic resistance gene, were also present in the plasmid DNA and were therefore also subsequently transferred to the soybeans. These genetic elements are described in Table 2 below.

Table 2: Description of other genetic elements transferred to high oleic acid soybeans

Genetic element	Source	Function
lac	An incomplete copy of the *lac* operon which contains a partial *lac*I coding sequence, the promoter P_{lac}, and a partial coding sequence for β-D-galactosidase (*lac*Z′).	These genes are not intact and no longer function in *E.coli.*
ori	Origin of replication from the high copy number *E. coli* plasmid pUC19.	Allows plasmids to replicate in *E. coli.*
bla	Gene coding for the enzyme β-lactamase from *E. coli.*	Confers ampicillin resistance to *E. coli.*
f1 ori	Bacteriophage f1 origin of replication.	Origin of replication recognised by bacteriophage f1 to produce single stranded DNA. The f1 origin is not recognised unless a phage f1 is present.
***lac*Z′ 3'**	The 3' end of the *lac*Z′ gene.	This gene is non-functional and no longer intact.

The bacterial *bla* gene is a marker used to select transformed bacteria from non-transformed bacteria during the DNA cloning and recombination steps undertaken in the laboratory prior to transformation of the plant cells. It codes for the enzyme β-lactamase and confers resistance to some β-lactam antibiotics, such as penicillin and ampicillin. Only those bacterial cells that have been transformed with the plasmid containing the *bla* gene, and hence the gene

of interest (in this case, the *GmFad 2-1* gene) will grow. The *bla* gene is under the control of a bacterial promoter and is therefore not expressed in transformed plant cells.

The remaining genetic elements are present in most *E. coli* cloning vectors and are well described (Sambrook *et al* 1981). They are used to assist in the manipulation of DNA sequences as well as direct gene expression in *E.coli.*

2.3 Characterisation of the genes in the plant

Selection of plant lines

From the initial population of transformed plants, one plant (Event 260-05) was selected which exhibited GUS activity and which was also shown, using the polymerase chain reaction (PCR), to contain the *GmFad 2-1* gene. Small samples were taken from the R1 seeds of plant 260-05 and screened for fatty acid composition and lysine content. Four different fatty acid profiles in combination with lysine changes were identified among the R1 seeds:

(i) seeds with ≥ 80% oleic acid content and normal lysine levels (G168);

(ii) seeds with about 72% oleic acid content and increased lysine levels (G94);

(iii) seeds with about 4% oleic acid content and increased lysine levels (G175); and

(iv) seeds with oleic acid and lysine levels similar to that of line A2396 (G90).

R2 seeds from (i) and (ii) above were further characterised by fatty acid composition and lysine assays. Southern blot analysis was also done on R1 and R2 leaves.

Southern blotting is a sensitive technique that enables the detection of specific sequences among DNA fragments that have been separated using gel electrophoresis (Southern 1975). . The overall pattern of the specific fragments detected can be used to characterise the nature of the T-DNA insertion into the genome (e.g. how many loci in the genome has the T-DNA have inserted into, whether the inserted copies are intact, etc).

Southern blotting of genomic DNA extracted from R1 and R2 leaves revealed that the *GmFad 2-1* construct had become integrated at two different loci in the genome of the original transformant (line 260-05). At one locus (*locus A*), the *GmFad 2-1* construct was causing silencing of the endogenous *GmFad 2-1* gene, resulting in seeds like G168 with a high oleic acid content only. *Locus A* was characterised using Southern blotting and shown to contain two copies of the *GmFad 2-1* expression cassette as indicated by two hybridising bands on the Southern blot. The second locus (*locus B*) contained a copy of *GmFad 2-1* that was over-expressing, thus decreasing the oleic acid levels to around 4% (G175). This locus also contained a functioning *dapA* gene as evidenced by an increase in the seed lysine levels. *Locus B* contained only a single copy of the *GmFad 2-1* expression cassette as indicated by a single hybridising band on the Southern blot. In seeds with both *locus A* and *locus B* (G94), oleic acid levels were increased but not as high as *locus A* alone and lysine levels were increased.

Lines G94 and G168 were selected for further characterisation as they contained the silencing *locus A* with the high oleic acid phenotype. As G94 plants contained both *locus A* and *locus*

B, an additional round of selection was used on the segregating R2 plants to isolate plants containing *locus A* and not *locus B*. Southern blot analysis on R2 leaf tissue grown from G94 R2 seed identified two sub lines, G94-1 and G94-19, that contained *locus A* without *locus B* which had been removed through segregation. *Locus B* was not further characterised for the purposes of this application.

The three sub lines, G94-1, G94-19 and G168, identified as containing the *GmFad 2-1* silencing *locus A*, were selected as the high oleic acid soybeans for subsequent analyses. The application for food use relates to these sub lines only.

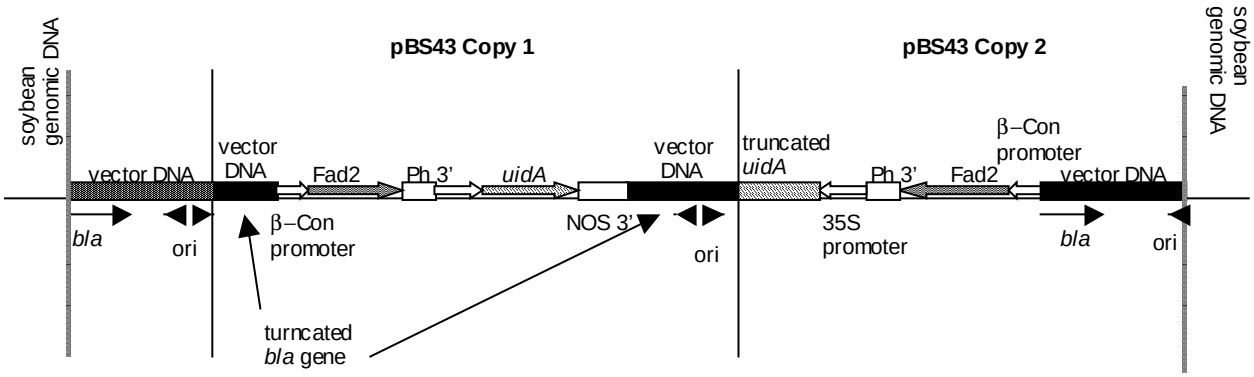
Molecular characterisation of the gene insertion in sub lines G94-1, G94-19 and G168

Studies submitted by Optimum Quality Grains:

Luckring, A. *et al* (1999). Southern blot analysis of high oleic soybean sublines G94-1, G94-19 and G168 derived from Event 260-05. Optimum Quality Grains Study No. 99 Southern-HOS-02.

A set of six different DNA hybridisation probes, specific for different parts of the *GmFad 2-1* expression cassette, were used to fully characterise and map the DNA insertion at *locus A* of R6 leaf tissue.

The mapping of *locus A* shows that one copy of pBS43, opened in the *bla* gene, inserted intact into the genome. A second copy of pBS43, opened in the *uidA* gene, inserted as an inverted repeat relative to the first copy. At the 5' end of *locus A*, proceeding from the soybean genomic DNA junction to the first copy of pBS43, a fragment of pML102, containing only the vector region with the *bla* gene, was inserted. Therefore, the insertion at *locus A* consists of two intact copies of the *GmFad 2-1* expression cassette, one intact copy of the *uidA* expression cassette and a truncated copy of the *uidA* gene, and at least two intact copies of the *bla* gene plus one truncated copy. A diagram of the gene organisation at *locus A* is presented in below.



A series of Northern blots (for RNA expression), Western blots (for protein expression) and amino acid profiles were done on sub lines G94-1, G94-19 and G168 to confirm that the functional *dapA* gene at *locus B* was absent. However, additional Southern blots, using a *dapA* probe, indicated that a truncated *dapA* gene expression cassette had become integrated into another locus in the genome (*locus C*). This locus segregates independently of *locus A*.

The truncated *dapA* gene is non-functional as indicated by Northern, Western and amino acid analyses.

2.4 Stability of the genetic changes

Sub lines G94-1, G94-19 and G168 differ from the parent line A2396 in that the fatty acid profile has been altered to produce oil containing about 82-85% oleic acid with consequent low levels of linoleic (< 1%) and linolenic acids (< 2.5%). This compares to a range of 19 – 30% oleic acid reported for standard edible soybean oil (Codex Alimentarius 1989).

To evaluate the genetic and phenotypic stability of the sub lines, genomic DNA from a number of generations of high oleic acid soybeans, homozygous for the *GmFad 2-1* silencing *locus A,* were subject to detailed Southern blot analyses. The applicant reports that sub lines G94-1, G94-19 and G168 had been kept separate for six generations and all were shown to maintain identical Southern banding patterns over that period. Analysis of the oleic acid content of seeds from eight different generations also showed that the fatty acid phenotype was stable over this period, with average oleic acid content greater than 80%. In addition, the high oleic acid trait is also reported by the applicant to be stable over a number of different growing environments when compared to the elite parent line and a high oleic acid soybean line derived through conventional breeding methods.

Conclusion

The inserted genes in the three sub lines of high oleic acid soybeans are stably integrated and all three lines are phenotypically and genetically stable over multiple generations and in various environments.

3. GENERAL SAFETY ISSUES

The high oleic acid soybeans have been assessed according to ANZFA's safety assessment guidelines relating to Group D foods, i.e., plants or animals that contain new or altered genetic material (ANZFA 1999).

3.1 History of use

Soybean (*Glycine max*.), which is grown as a commercial crop in over 35 countries worldwide, has a long history of safe use for both human food and stockfeed. The elite soybean cultivar A2396, which has been used as the host for the high oleic acid trait described in this application, is an Asgrow Seed Company early Group II maturity soybean variety that has high yield potential. Protein and oil characteristics are said to be similar to other soybeans at 40% protein and 22% oil on a dry weight basis.

There are three major soybean commodity products: seeds, oil and meal. There is only limited feed use, and no food use, for unprocessed soybeans, as they contain toxicants and anti-nutritional factors, such as lectins and trypsin inhibitors. Appropriate heat processing inactivates these compounds. Whole soybeans are used to produce soy sprouts, baked soybeans, and roasted soybeans. The soybean hulls can be processed to create full fat soy flour and the traditional soy foods such as miso, tofu, soymilk and soy sauce. Before processing, soybeans are graded, cleaned, dried and de-hulled. The soybean hulls are further processed to create fibre additives for breads, cereals and snacks and are also used for

stockfeed. After de-hulling, soybeans are rolled into full fat flakes that may be either used in stockfeed or processed further into full fat flour. Crude soybean oil is then extracted from the flakes by immersing them in a solvent bath. Crude lecithin is then separated from the oil, which is further refined to produce cooking oil, margarine and shortening. After the oil is extracted from the flakes, the solvent is removed and the flakes are dried for use in the production of soy flour, soy concentrates and soy isolates. De-fatted soy flakes are also used in stockfeed.

The applicant anticipates that high oleic acid soybean oil will be predominantly used in spraying and frying applications in the food industry and food services and might replace heat stable fats and oils such as hydrogenated soybean and rapeseed oil or palm olein/vegetable oil blends.

3.2 Nature of novel protein

See section 3.3 below.

3.3 Expression of novel protein in the plant

Studies submitted by Optimum Quality Grains:

Stecca, K. (1996). Northern blot analysis of high oleic soybean sublines G94-1, G94-19 and G168 derived from Event 260-05. Optimum Quality Grains Study No. 96 Northern-HOS-33.

Sanders, C. (1997). Analysis of protein expression in high oleic soybean sublines G94-1, G94-19 and G168 derived from Event 260-05 by enzyme assays, western blots and protein gel electrophoresis. Optimum Quality Grains Study No. 97 Protein-HOS-02.

δ-12 desaturase

Northern blot analysis, using the *GmFad 2-1* gene as a probe, was done on RNA isolated from developing R4 seeds of the high oleic acid soybeans at the time when the endogenous *GmFad 2-1* would normally be expressed (during seed development, 20 days after flowering). The β-conglycinin promoter, linked to the transferred copy of the *GmFad 2-1* gene, is also active during this period. The data shows that seeds containing *GmFad 2-1* silencing *locus A* (G94-1, G168) do not have any detectable *GmFad 2-1* mRNA, whereas, seeds that contain the *GmFad 2-1* over expressing *locus B* (G175) or seeds that only contain the endogenous *GmFad 2-1* gene (G90) have significant levels of mRNA. This demonstrates that neither of the *GmFad 2-1* genes is transcribed in the high oleic acid soybeans.

Dihydrodipicolinic acid synthase

Northern blot analysis, using the *dapA* probe, was done on RNA isolated from R6 leaves and R4 immature seeds of the high oleic acid soybeans. The data show that there is no detectable expression of *dapA* mRNA in sub lines G94-1, G94-19 and G168. Western blot analysis, using a polyclonal anti-*Corynebacterium* DHDPS antibody, was done on total protein isolated from leaves and seeds of the three sub lines. The data show that DHDPS protein can only be detected in seeds of the high lysine positive control line and not in any of the high oleic acid sub lines under consideration.

Amino acid analyses were done on three replicates of each of the high oleic acid soybean sub lines. These show that there are no differences in the lysine levels of the high oleic acid soybeans when compared to the parental soybean line (A2396).

β-glucuronidase

An intact *uidA* expression cassette is present in sub lines G94-1, G94-19 and G168, however, colourimetric analyses of R6 seeds and leaves from these lines show that the *uidA* gene is not expressed. The original transformant, line 260-05, was selected on the basis of its GUS expression therefore the *uidA* gene has become 'switched off' in subsequent generations. The applicant has not speculated as to the reason for the inactivation of the *uidA* gene, however, the inactivation of transgenes is relatively common in plants (Kilby *et al* 1992, Ingelbrecht *et al* 1994, Brusslan and Tobin 1995).

β-lactamase

All of the lines derived from event 260-05, which contain only the *GmFad 2-1* silencing *locus A,* also contain two intact copies of the *bla* gene. These two copies are under the control of a bacterial promoter and, therefore, should not be expressed in the plant cell. To confirm this, the activity of β-lactamase was measured in cell free extracts of leaf tissue from sub line G94-1. The results of this study, which show that there is no detectable β-lactamase activity in sub line G94-1, confirm that the *bla* gene is not expressed in plant cells.

Conclusion

There are no novel proteins expressed in high oleic acid soybean sub lines G94-1, G94-19 and G168.

3.4 Impact on human health from potential transfer of novel genetic material to cells in the human digestive tract

The human health considerations in this regard depend on the nature of the novel genes and must be assessed on a case-by case basis.

In 1991, the World Health Organization (WHO) issued a report of a Joint FAO[4]/WHO Expert Consultation which looked at strategies for assessing the safety of foods produced by biotechnology (WHO 1991). It was concluded by that consultation that as DNA from all living organisms is structurally similar, the presence of transferred DNA in food products, in itself, poses no health risk to consumers.

The major concern in relation to the transfer of novel genetic material to cells in the human digestive tract is with antibiotic resistance genes. Antibiotic resistance genes can be present in some transgenic plants as a result of their use as marker genes to select transformed cells. It is generally accepted that there are no safety concerns with regard to the presence in the food of antibiotic resistance gene DNA *per se* (WHO 1993). There have been concerns expressed, however, that there could be horizontal gene transfer of antibiotic resistance genes from ingested food to microorganisms present in the human digestive tract and that this could compromise the therapeutic use of antibiotics.

[4] Food and Agriculture Organization.

This section of the report will therefore concentrate on evaluating the human health impact of the potential transfer of antibiotic resistance genes from high oleic acid soybeans to microorganisms present in the human digestive tract.

The two plasmids used to transform soybean line A2396 - pBS43 and pML102 - both contained a copy of the *bla* gene under the control of a bacterial promoter. The *bla* gene encodes the enzyme β-lactamase and confers resistance to a number of β-lactam antibiotics such as penicillin and ampicillin. Analysis of the high oleic acid soybean lines has confirmed the presence of two intact copies of the *bla* gene along with its bacterial promoter. The *bla* gene is not itself expressed in the high oleic acid soybean lines.

The first issue that must be considered in relation to the presence of an intact *bla* gene in the high oleic acid soybeans is the probability that this gene would be successfully transferred to and expressed in microorganisms present in the human digestive tract. The following steps are necessary for this to occur:

- excision of DNA fragments containing the *bla* gene and its bacterial promoter;
- survival of DNA fragments containing the *bla* gene in the digestive tract;
- natural transformation of bacteria inhabiting the digestive tract;
- survival of the bacterial restriction system by the DNA fragment containing the *bla* gene;
- stable integration of the DNA fragment containing the *bla* gene into the bacterial chromosome or plasmid;
- maintenance and expression of *bla* gene by the bacteria.

The transfer of a functional *bla* gene to microorganisms in the human digestive tract is therefore highly unlikely because of the number and complexity of the steps that would need to take place consecutively.

The second and most important issue that must be considered is the potential impact on human health in the unlikely event successful transfer of a functional *bla* gene to microorganisms in the human digestive tract did occur.

In the case of transfer of the *bla* gene from high oleic acid soybeans to microorganisms of the digestive tract, the human health impacts are considered to be negligible. This is because ampicillin-resistant bacteria are commonly found in the digestive tract of healthy individuals (Calva *et al* 1996) as well as diseased patients (Neu 1992). Therefore, the additive effect of a *bla* gene from the high oleic acid soybeans being taken up and expressed by microorganisms of the human digestive tract would be insignificant compared to the population of ampicillin resistant bacteria already naturally present.

In relation to transfer of novel genetic material from genetically modified food to human cells via the digestive tract, this is also equally unlikely to occur. In considering the potential impact on human health, it is important to note that humans have always consumed large amounts of DNA as a normal component of food and there is no evidence that this

consumption has had any adverse effect on human health. Furthermore, current scientific knowledge has not revealed any DNA sequences from ingested foods that have been incorporated into human DNA. Novel DNA sequences in genetically modified foods comprise only a minute fraction of the total DNA in the food (generally less than 0.01%) and are therefore unlikely to pose any special additional risks compared with the large amount of DNA naturally present in all foods.

Conclusion

It is extremely unlikely that the ampicillin resistance gene will transfer from high oleic acid soybeans to bacteria in the human digestive tract because of the number and complexity of steps that would need to take place consecutively. In the highly unlikely event that the ampicillin resistance gene was transferred to bacteria in the human digestive tract the human health impacts would be negligible because ampicillin resistant bacteria are already commonly found in the human gut and in the environment.

It is also equally unlikely that novel genetic material from the high oleic acid soybeans will be transferred to human cells via the digestive tract. The novel genetic material in the high oleic acid soybeans comprises only a minute fraction of the total DNA in the soybeans therefore it is unlikely to pose any special additional risks compared with the large amount of DNA naturally present in all foods.

4. TOXICOLOGICAL ISSUES

4.1 Levels of naturally occurring toxins

Studies submitted by Optimum Quality Grains:

Anon (1996). Compositional analysis of high oleic soybean sub lines G94-1, G94-19 and G168 derived from Event 260-05. Optimum Quality Grains Study No. 96 Field-Comp-HOS-11.

The only naturally occurring toxins in soybeans are lectins. Lectins are proteins that bind to carbohydrate-containing molecules and which inhibit growth and sometimes cause death in animals. It is reasonable to assume that similar effects would occur in humans. Lectins, however, are rapidly degraded upon heating, and therefore only become an issue when raw soybeans are consumed. There are no human food uses for raw soybeans.

Notwithstanding that there are no human food uses for raw soybeans, the applicant undertook compositional analyses for lectin content of seeds from the high oleic acid soybean lines. The seeds represent the R6 generation of the high oleic acid soybean lines. Lines G94-1, G94-19 and G168 were grown in parallel with the parental line A2396 at four locations in the United States in the summer of 1996. To obtain the data, three replicates were analysed in duplicate from each of the four locations. The results of these analyses are summarised in Table 3 below.

Table 3: Lectin content[#] of parental and high oleic acid soybean lines

Lectin	Parental control	High oleic soybeans	Literature range
HU[1]/mg extracted protein	6.36 (4.09-7.90)	7.83 (5.37-9.70)	2.7-12.5
HU/mg total protein	2.98 (2.30-3.90)	3.67 (2.77-4.73)	1.2-6.0
HU/mg sample (FW basis)	1.03 (0.70-1.30)	1.32 (0.97-1.67)	0.5-2.4

[1] HU = haemagglutinating unit

mean values, the range in brackets

The high oleic acid soybean lines exhibit slightly elevated lectin levels when compared to the control. The values reported however are well within the literature reported range for soybeans. As lectins are readily degraded upon heating, and the levels reported are still within the literature reported range, the slightly elevated levels do not represent a safety concern.

4.2 Potential toxicity of novel protein

Detailed Northern and protein analyses have demonstrated that no new proteins are expressed in sub lines G94-1, G94-19 and G168. Therefore, there are no toxicological issues to consider in relation to any new proteins.

4.3 Levels of naturally occurring allergenic proteins

Study submitted by Optimum Quality Grains:

Lehrer, S. (1996). Allergenicity of high oleic acid soybeans. Tulane University School of Medicine, Section of Allergy and Clinical Immunology, New Orleans, LA, USA.

The protein profile of the high oleic acid soybeans was found to be different in a number of respects to that of the parental soybean line A2396.

Soybean 7S and 11S globulins are two major storage proteins accounting for about 70% of total meal protein. The 7S fraction is made up of the α, α^1, and β subunits of β-conglycinin. The 11S fraction is made up of the acidic (A) and basic (B) subunits of glycinin. The high oleic acid soybeans were found to have reduced concentrations of the α and α^1 subunits of β-conglycinin, when compared with the parental A2396 soybean lines. This was coincident with an increase in the concentration of the A and B subunits of glycinin in addition to an increase in the concentration of the A2B1A glycinin precursor. The profile of other storage proteins appears to be identical to that of A2396.

The applicant speculates that the reduction in concentration of the β-conglycinin α and α^1 subunits is due to co-suppression by the α^1 promoter sequence used in the *GmFad 2-1* vector (pBS43). The phenomenon of co-suppression has been observed for other genes and plants and is well documented in the literature (Brusslan and Tobin, 1995).

A study was done to determine whether alterations to the protein profile of the high oleic soybeans had changed their allergenicity relative to the parental soybean line.

Radioallergosorbent (RAST) reactivity

Extracts were made of the parental soybean line A2396 and high oleic acid soybean line G94-1. Sera were used from 31 subjects with a history of documented soybean or food allergy, a positive skin test to soybean extract, and/or a positive IgE antibody response to soybean extract. Control sera were obtained from soybean tolerant individuals with a negative skin test and/or RAST to soy extract with total IgE levels similar to those sera of soybean-sensitive subjects.

In RAST reactivity assays many of the sera demonstrated significant IgE antibody reactivity to soybean extracts. Twenty-one of the 31 sera tested had IgE antibody % binding greater

than or equal to 4 %. Eleven of the 21 positive sera had IgE antibody binding in excess of 20%. The sera with the most significant RAST reactivity were pooled for RAST inhibition studies.

RAST inhibition

Both the parental and high oleic acid soybean extracts yielded virtually identical RAST inhibition curves to the parental soybean RAST.

Immunoblot analysis

The 21 most potent RAST positive sera were selected for immunoblot analyses of soybean allergens. The immunoblot analysis showed, as expected, that there are a number of proteins in the soybean extract that bind IgE antibodies from soybean allergic sera. Some sera were more reactive than others, so six of the most reactive sera were selected and pooled for further study of the allergens present in the parental and high oleic acid soybeans. Both colourimetric and chemiluminescence techniques were used for the detection of reactive protein bands.

The results show that there are no significant differences in the number of protein bands to which the sera react or to the intensity of the IgE reactivity.

Conclusion

There are no significant differences in the allergen content of the high oleic acid soybeans compared to the parental soybean line A2396.

4.4 Potential allergenicity of novel proteins

There are no novel proteins expressed in the high oleic acid soybeans.

5. NUTRITIONAL ISSUES

5.1 Nutrient analysis

There are concerns that transformation will affect the overall nutritional composition of a food, or cause unintended changes that could adversely affect the safety of the product. Therefore a safety assessment of food produced from transgenic plants must include analysis of the composition of the food, based on a comparison with other commercial varieties of the crop. Generally, comparisons are made not only with the parental line but also with other non-transformed lines. If the parameter for the transformed line is within the normal range for non-transformed lines, this is considered acceptable (Hammond and Fuchs 1998).

Studies submitted by Optimum Quality Grains:

Anon (1996). Compositional analysis of high oleic soybean sub lines G94-1, G94-19 and G168 derived from Event 260-05. Optimum Quality Grains Study No. 96 Field-Comp-HOS-11.

The applicant undertook two separate field studies of the high oleic acid soybeans. In the first study, lines G94-1 and G94-19 were grown at two locations in the United States: Slater, Iowa, and Isabella, Puerto Rico during the summer of 1995 and the Winter of 1995/1996. Seeds, representing the R4 and R5 generation, were analysed from each location. Values were

obtained from duplicate assays on single samples from each of the four locations. Analyses were done of raffinose, stacchyose and phytic acid content as well as isoflavone content. In the second study conducted in the summer of 1996, lines G94-1, G94-19 and G168 were grown in parallel with the parental line A2396 at four locations in the United States: Redwood Falls, Minnesota, Kalamazoo, Michigan, Prairie City, Iowa and Cedar Rapids, Iowa. Seeds, representing the R6 generation, were analysed from each of the four locations. Values were obtained from duplicate assays on three replicates from each of the four locations. Analyses were done of proximate, trypsin inhibitor, amino acid, fatty acid, vitamin and mineral, and tocopherol content.

Proximate analyses

Proximate analysis includes the measurement of crude fat/oil, protein, fibre, and ash content and is done to determine if there have been any changes to the primary constituents of the soybean seed. The results of the proximate analysis are presented in Table 4 below.

Table 4: Proximate content[#] of control and high oleic acid soybeans

	Parental control	High oleic lines	Literature range
	(g/100 g dry weight unless noted)		
Moisture (g/100 g fresh wt)	7.69 (7.00-8.20)	7.85 (7.20-8.40)	7-11
Crude fat/oil	25.37 (21.62-28.29)	23.90 (19.74-29.28)	13.2-22.5
Protein	40.11 (38.41-41.68)	40.76 (38.85-42.97)	36.9-46.4
Fibre	6.11 (5.44-7.14)	6.76 (5.00-7.26)	4.7-6.8
Ash	5.13 (4.53-5.85)	4.81 (4.13-5.54)	4.61-5.37

[#] mean values, the range in brackets

The results show that there are no significant differences in proximate composition between the parental soybean line and the high oleic acid soybeans. The values obtained are also comparable to those reported in the literature for soybeans.

Amino acid composition

Amino acid content was determined for 17 out of the 20 amino acids. The three amino acids not analysed were proline, asparagine and glutamine. A summary of the results of the amino acid analysis appears in Table 5 below.

Table 5: Amino acid content[#] of parental and high oleic acid soybeans

Amino acid	Parental control	High oleic lines	Literature range
		(g/100 g dry weight)	
Tryptophan	0.44 (0.41-0.46)	0.47 (0.42-0.51)	0.53-0.54
Lysine	2.45 (2.27-2.63)	2.38 (2.17-2.67)	2.35-2.86
Histidine	0.96 (0.90-1.05)	0.93 (0.83-1.09)	0.89-1.08
Arginine	2.64 (2.42-2.91)	2.64 (2.37-2.88)	2.45-3.49
Aspartic acid	4.3 (3.98-4.58)	4.45 (4.14-4.93)	3.87-4.98
Threonine	1.37 (1.24-1.50)	1.52 (1.38-1.70)	1.33-1.79
Serine	1.79 (1.61-1.95)	1.84 (1.65-2.02)	1.81-2.32
Glutamic acid	7.13 (6.58-7.81)	7.03 (6.50-7.79)	6.10-8.72
Cysteine	0.55 (0.51-0.60)	0.58 (0.52-0.71)	0.56-0.66
Glycine	1.57 (1.44-1.68)	1.71 (1.56-1.85)	1.88-2.02
Alanine	1.54 (1.43-1.68)	1.67 (1.50-1.84)	1.49-1.87
Valine	1.73 (1.61-1.86)	1.84 (1.58-2.05)	1.52-2.24
Methionine	0.47 (0.44-0.50)	0.54 (0.47-0.60)	0.49-0.66
Isoleucine	1.72 (1.48-1.87)	1.76 (1.54-2.00)	1.46-2.12
Leucine	2.86 (2.64-3.05)	2.91 (2.70-3.18)	2.71-3.20
Tyrosine	1.45 (1.35-1.54)	1.51 (1.38-1.62)	1.12-1.62
Phenylalanine	1.82 (1.71-1.97)	1.86 (1.72-2.03)	1.70-2.08

[#] mean values, the range in brackets

No significant differences were observed in amino acid content between the parental line and the high oleic acid soybeans for any of the 17 amino acids analysed. The values determined were comparable to the literature reported ranges.

Fatty acid composition

A complete fatty acid analysis of oil from the high oleic acid soybean lines G94-1 and G94-19 and control soybean lines grown in field trials in 1995/1996 was done and compared to the ranges specified by Codex Alimentarius for soybean oil. The results of the analysis are presented in Table 6 below.

Table 6: Complete fatty acid analysis of control and high oleic acid soybean lines from 1995/96 field trials

Fatty acid	Parental control	G94-1	G94-19	Codex range
	(g/100 g fatty acid, mean values presented, ranges not provided)			
C14:0 myristic	<0.1	<0.1	<0.1	<0.5
C16:0 palmitic	10.1	6.3[#]	6.6	7.0-14.0
C16:1 palmitoleic	0.1	0.12	0.12	<0.5
C16:2 hexadienoic	<0.1	<0.1	<0.1	
C16:3 hexatrienoic	<0.1	<0.1	<0.1	
C18:0 stearic	3.2	3.7	3.6	1.4-5.5
C18:1 oleic	14.7	84.6	84.9	19.0-30.0
C18:2 (9,12) linoleic	61.6	0.9	0.6	44.0-62.0
C18:2 (9, 15) linoleic	<0.1	0.8	0.7	
C18:3 linolenic	9.5	2.4	1.9	4.0-11.0
C20:0 arachidic	0.2	0.4	0.5	<0.1
C20:1 eicosenoic	0.2	0.4	0.4	<0.1
C20:2 eicosadienoic	not done	not done	not done	
C22:0 behenic	0.3	0.4	0.5	<0.5
C22:1 erucic	<0.1	<0.1	<0.1	
C24:0 lignoceric	0.1	0.1	0.2	

[#] Underlined values are significantly different from the parental control

A further, but more limited analysis of fatty acid content was done on all three high oleic acid soybean lines and the parental control soybean line grown in field trials in 1996. The results of the analysis are presented in Table 7 below.

Table 7: Fatty acid composition[#] of oil from high oleic acid and control soybean lines from 1996 field trials

Fatty acid	Parental control	High oleic lines	Literature Range
		(g/100 g fatty acid)	
C16:0 palmitic	10.25 (9.94-10.59)	6.55 (6.22-6.96)	7-12
C18:0 stearic	3.95 (3.57-4.27)	3.43 (3.04-3.81)	2-5.5
C18:1 oleic	23.09 (22.07-23.91)	83.84 (80.02-85.38)	20-50
C18:2 linoleic	55.36 (53.61-56.48)	2.23 (1.19-4.83)	35-60
C18:2 9,15 linoleic isomer	0.00	0.48 (0.37-0.56)	-
C18:3 linolenic	7.35 (6.81-8.35)	3.47 (2.87-4.51)	2-13

[#] mean values, the range in brackets

The results from the two separate analyses demonstrate that the high oleic acid soybeans differ significantly from the parental soybean line in the levels of oleic, linoleic, linolenic and palmitic acid present in the oil. Oleic acid levels have been significantly increased and this has resulted in concomitant decreases in the levels of palmitic, linoleic and linolenic acids. The levels of other fatty acids present in the oil were similar between the parental and high oleic acid soybean lines and were comparable to the Codex Alimentarius ranges for soybean oil. High levels of oleic acid are commonly consumed in other premium edible oils (e.g.,

olive oil, high oleic sunflower and canola oils). The increased oleic acid levels do not pose a safety concern.

In addition to the expected changes to the fatty acid composition of oil from the high oleic acid soybean lines, a trace amount (less than 1% of the total fatty acid content) of the 9,15 isomer of linoleic acid (cis-9, cis-15-octadecadeinoic acid), normally found only in hydrogenated soybean oils and butterfat, was also detected. This isomer is not present in the oil of the parental soybean line A2396.

The applicant speculates that the presence of the isomer is the result of activity of a δ-15 (n-3) desaturase (*GmFad3*), which normally inserts a δ-15 double bond into 9,12-linoleic acid. In the transgenic plants, the linoleic acid content is reduced from >50% of the total fatty acids to <2% and therefore they speculate that the *GmFad3* enzyme probably creates a small amount of the isomer by putting a δ-15 double bond into 9-oleic acid. The applicant provided data to support this hypothesis where the high oleic acid soybeans were crossed with a soybean containing a suppressed *GmFad3* gene. In the resulting progeny, the isomer is either reduced or virtually eliminated.

The applicant provided data on the occurrence of the 9,15 isomer of linoleic acid in commonly used oils and fats for frying and baking in Europe. This data is presented in Table 8 below.

Table 8: Occurrence of the 9,15 linoleic acid isomer in commonly used oils and fats for frying and baking

	Fatty acid composition (g/ 100 g fatty acid)					
Oil/fat	**C16:0**	**C18:0**	**C18:1**	**C18:2**	**C18:2 (9,15)**	**C18:3**
Palm olein, partially hydrogenated	20.8	4.0	48.3	22.4	1.3	0.8
Soybean oil, partially hydrogenated	10.8	5.8	44.8	21.4	3.4	0.7
Rapeseed oil, partially hydrogenated	5.6	3.8	72.0	8.9	2.7	1.3
Butter fat	34.8	11.7	26.6	2.6	0.4	0.8

This data shows that the 9,15 isomer of linoleic acid is commonly found in other edible sources of fat such as butterfat and partially hydrogenated vegetable oils at a range of 0.4-3.4% of the total fatty acids. Therefore, its occurrence in high oleic acid soybean oil at a level of 0.5% of the total fatty acids (representing about 25% of the linoleic acid fraction) is not considered to pose any safety concerns.

Vitamins and minerals

The high oleic acid soybean lines G94-1, G94-19 and G168 and the parental soybean line A2396 were analysed for their mineral and vitamin content including tocopherols. The tocopherols, also known as vitamin E, exist as four isomers (α-, β-, γ-, and δ-tocopherol). The four isomers are not equivalent, with α-tocopherol being the most important in terms of bioactivity. The Recommended Daily Intake (RDI) for vitamin E is normally presented as α-tocopherol equivalents. The results of the vitamin and mineral analyses are summarised in Table 9 below.

Table 9: Vitamin and mineral content[*] of the control and high oleic acid soybeans

Vitamin or mineral[#]	Parental control	High oleic lines	Literature range
	(mg/100 g dry weight unless noted)		
Minerals:			
Calcium	264 (245-302)	232 (212-251)	132.7-326.3
Copper	0.64 (0.30-1.00)	0.67 (0.24-1.02)	0.9-5.1
Iron	5.6 (4.2-7.4)	5.8 (3.8-7.9)	3.2-7.9
Magnesium	247 (232-260)	236 (215-261)	
Manganese	2.9 (1.9-4.0)	2.7 (2.2-3.6)	0.4-6.8
Phosphorous	621 (516-742)	636 (501-771)	378-1836
Potassium	1755 (1468-1950)	1689 (1492-1896)	859-1784
Sodium	3.1 (1.1-6.5)	4.3 (2.2-8.7)	
Zinc	4.0 (3.2-4.7)	4.3 (3.0-5.8)	
Vitamins:			
Vitamin B6	0.115 (0.098-0.131)	0.125 (0.110-0.141)	
β-carotene (IU/100 g dry wt)	8 (5-12)	10 (5-16)	
Vitamin B1	0.96 (0.74-1.17)	0.89 (0.63-1.24)	
Vitamin B2	0.29 (0.26-0.30)	0.30 (0.27-0.35)	
Vitamin E (IU/100 g dry wt)	1.2 (1.1-1.6)	1.1 (0.9-1.7)	
Niacin	2.6 (2.28-2.88)	2.74 (2.38-3.15)	
Pantothenic acid	1.051 (0.936-1.132)	0.961 (0.794-1.063)	
Folic acid (μg/100 g dry wt)	274 (184-379)	284 (186-384)	
Tocopherols:			
Total	20.11 (18.01-22.50)	18.57 (16.36-21.16)	
alpha	1.37 (1.11-1.62)	1.32 (1.06-1.62)	1.09-2.84
beta	0.17 (0.07-0.20)	0.22 (0.15-0.30)	<0.5
gamma	16.17 (14.03-18.81)	15.42 (13.12-17.58)	15.0-19.1
delta	1.72 (1.52-2.11)	1.88 (1.61-2.28)	2.46-7.25

[#] all samples contained less than 0.1 μg/100 g vitamin B12, less than 1.0 mg/100 g vitamin C and less than 5 IU/100 g retinol

[*] mean values, the range in brackets.

No significant differences in mineral or vitamin content, including tocopherols, were observed between the high oleic acid soybeans and the parental soybean line. The mineral content of the high oleic acid soybeans was within the literature reported ranges. With the exception of the tocopherols, literature ranges for vitamin content was not provided. The delta tocopherol content was lower than the literature reported range for both the parental control and high oleic acid soybean lines. The content of the other tocopherols in the high oleic acid soybeans were within the literature reported ranges for soybeans.

Isoflavones

Soybeans naturally contain a number of isoflavone compounds reported to possess biochemical activity, including estrogenic and hypocholesterolemic effects, in mammalian species. Isoflavones (known to include phytoestrogens) have, in the past, also been regarded as anti-nutrients, however, this is no longer universally accepted as isoflavones have also been reported to have beneficial anti-carcinogenic effects. The major isoflavones in soybeans and soybean products include daidzin, genistin, and their corresponding aglycons, daidzein and genistein. Glycitin and glycitein also occur in trace amounts.

High oleic acid soybean lines G94-1 and G94-19 and parental soybean line A2396 were analysed for isoflavone content. The results are summarised in Table 10 below.

Table 10: Isoflavone content[#] of parental and high oleic acid soybean lines

Isoflavone	Parental control	High oleic lines	Literature range
		(µg/g dry weight)	
Total daidzein	693 (623-762)	612 (525-694)	295-1527
Total genistein	714 (574-854)	724 (548-910)	416-2676
Total glycitein	192 (188-196)	273 (261-287)	149-341

[#] mean values, range in brackets

There are no significant differences between the parental soybean and the high oleic acid soybean lines G94-1 and G94-19 in either total daidzein or genistein content which is also within the literature reported ranges for soybeans. In relation to total glycitein content, however, the high oleic acid soybean lines exhibit slightly elevated levels compared to the control. The level reported for total glycitein however is within the literature reported range therefore this slightly elevated level compared to the control is not considered to pose any safety concerns.

Other constituents

The fermentable galacto-oligosaccharides, raffinose and stacchyose, are present in soybeans and can be responsible for the production of unpleasant side-effects, such as flatulence, when soybeans and soybean products are ingested. The processing of soybean flours into concentrates and isolates removes these oligosaccharides. Seeds representing the R4 and R5 generations of lines G94-1 and G94-19 were analysed for raffinose and stacchyose content. The results of the analyses are summarised in Table 11 below.

Table 11: Stacchyose and raffinose content[#] of parental and high oleic acid soybeans

Constituent	Parental control	High oleic soybean	Literature range
		(µmoles/g dry weight)	
Stacchyose	63 (60-67)	68 (65-75)	44.8-68.8
Raffinose	14 (14-14)	15 (14-16)	8.6-18.5

[#] mean values, the range in brackets

No significant differences were observed between the parental soybean line and the high oleic soybean lines for stacchyose and raffinose content. The values reported are comparable to the literature reported ranges.

5.2 Levels of anti-nutrients

Soybeans contain two well-described anti-nutritional factors. These are trypsin inhibitors and phytic acid. Trypsins inhibitors are heat labile anti-nutrients which interfere with the digestion of proteins and result in decreased animal growth. Because they are heat labile, however, they are destroyed during the processing of soy products by heat treatment. Phytic acid, on the other hand, remains stable through most soybean processing steps and has been implicated in interfering with the bioavailability of minerals such as calcium, magnesium and zinc.

Seed representing the R6 generation of lines G94-1, G94-19 and G168 were analysed for trypsin inhibitor and phytic acid content. The results are summarised in Table 12 below.

Table 12: Anti-nutrient content[#] for parental and high oleic acid soybeans

Anti-nutrient	Parental control	High oleic lines	Literature ranges
Trypsin inhibitor (TIU/mg dry wt)	31.67 (22.84-40.47)	30.20 (14.21-42.43)	26.4-93.2
Phytic acid (g/100 g dry wt)	1.42 (1.32-1.53)	1.42 (1.25-1.69)	1.3-4.1

[#] mean values, the range in brackets

No significant differences were observed between the parental soybean line and the high oleic soybean lines for either of the anti-nutrients. The values reported are comparable tos the literature reported ranges.

5.3 Ability to support typical growth and well-being

In assessing the safety of food produced using gene technology, a key factor is the need to establish that the food is nutritionally adequate and will support typical growth and well-being. 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. Where, on the basis of available data, there is still concern or doubt in this regard, carefully designed feeding studies in animals may provide further re-assurance that the food is nutritionally adequate. Such studies may be considered necessary where the compositional analysis indicates significant differences in a number of important components or nutrients or where there is concern that the bioavailability of key nutrients may be compromised by the nature of the genetic changes to the food.

In the case of the high oleic acid soybeans, the extent of the compositional and other data provided in the application is considered adequate to establish the safety of the food. Nonetheless, the applicant also provided two animal feeding studies to compare the wholesomeness of the high oleic acid soybeans with controls. Although not considered essential for establishing safety in this instance, these animal feeding studies have been reviewed as additional supporting data.

Studies submitted by Optimum Quality Grains:

Loughmiller, J.A. *et al* (1997). Influence of soybean meal variety and processing temperature on the growth performance of pigs from 25 to 45 lb. Animal Science Department, Kansas State University, Kansas, United States.

Araba, M. and Lohrmann, T.T. (1997). The effect of heat processing on two soy varieties as measured in growing broiler chicks. Poultry Science Department, University of Georgia, Athens, Georgia, United States.

Pig feeding study

This study was done to determine if soybean meal produced from high oleic acid soybeans would provide similar levels of growth performance in pigs as soybean meal from traditional varieties.

Three hundred and ninety (39/group) high-lean growth pigs (Newsham Hybrids) were fed diets consisting of processed soybean meal from either the high oleic acid soybean lines or a standard check-line soybean. The soybeans used to make the meal were processed at four different temperature ranges (80-85, 85-90, 90-95, 100-105 °C) under conditions that simulated commercial processing. Positive and negative control diets were made using commercially available soybean meal (46.5% crude protein). The positive control diet was formulated to contain dietary 1.3% lysine whereas the negative control diet was formulated to

contain 0.95% dietary lysine. All test diets also contained 0.95% lysine so that any differences in growth performance could be readily attributable to the processing temperature or the amino acid availability. All pigs were fed a common 3 stage diet series until being placed on the test diets at 21 days post weaning. All test diets were corn-soybean meal based and were fed until 38 days post weaning.

Growth performance of the pigs is indicated by the average daily gain (ADG) as well as the F/G ratio, which is a measure of the amount of the feed consumed (the average daily feed intake - ADFI) / ADG or, in other words, is an indication of how much food (in pounds) it takes to put on 1 lb of body weight in the animal. The F/G ratios obtained over the course of the study are provided in Table 13 below.

Table 13: Effect of soybean meal varieties and processing temperature on pig F/G ratios

	Day 0 to7	Day 7 to 14	Day 14 to 17	Day 0 to 17
Commercial meal:				
1.3% lysine	1.44	1.49	1.69	1.50
0.95% lysine	1.71	1.74	1.92	1.75
High oleic meal (0.95% lys):				
80-85°C	2.38	2.42	3.56	2.49
85-90°C	1.72	1.84	1.96	1.80
90-95°C	1.84	1.74	1.83	1.78
100-105°C	1.79	1.86	1.86	1.83
Check-line meal (0.95% lys):				
80-85°C	1.75	1.86	2.03	1.84
85-90°C	1.92	1.79	1.86	1.83
90-95°C	1.82	1.82	1.87	1.81
100-105°C	1.95	1.80	2.28	1.91

Pigs fed the positive control diet (commercially available soybean meal formulated to contain 1.3% dietary lysine) had increased performance (as measured by the ADG and the F/G ratio) than pigs fed any other treatment. This indicates that a dietary lysine content of 0.95% was insufficient to maximise growth performance of the pigs.

Pigs fed diets containing high oleic acid soybean meal were shown to have a similar growth performance compared to pigs fed diets containing either commercial soybean meal or meal derived from the check-line soybean formulated to similar lysine levels, when the high oleic acid soybean meal is processed at temperatures above 80-85 °C. The reason for the decreased performance, compared to the control, of pigs fed the high oleic acid soybeans processed at 80-85 °C is not readily apparent. The applicant speculates that the difference may be due to difficulties experienced with the processing of the soybeans in the pilot processing plant.

Chicken feeding study

This study was done to determine the effects of five different processing temperatures on the feeding value of the parental soybean line compared to the high oleic acid soybean lines.

Six hundred and sixteen (56/group) 1-day-old broiler chicks (Peterson x Arbor Acre) were randomly allotted to one of 11 dietary treatments. The chicks were fed diets consisting of soybean meal obtained from either a standard check-line soybean or the high oleic acid soybean lines and which had been processed at five different processing temperatures (raw, 80-85, 85-90, 90-95, and 100-105 °C). A positive control diet was included using commercially obtained high protein soybean meal. Test diets using the check-line soybean

meal or the high oleic acid soybean meal were formulated to meet all nutrient requirements except for the amino acid concentration. The positive control diet contained 23% crude protein and 1.2% lysine, while diets containing check-line or high oleic acid soybean meal contained 20% crude protein and 1.03% lysine. Growth performance was measured by daily weight gain, the feed conversion ratio (feed:gain), and final body weight. The results are summarised in Table 14 below.

Table 14: Effects of processing temperature and soybean meal source on chick performance

	Daily gain 0-18 d (g)	Feed intake 0-18 d (g)	Feed:gain 0-18 d (g)	Body weight 0-7 d (g)	Body weight 0-18 d (g)
Raw:					
Commercial	26.95	37.86	1.417	148.2	525.1
High oleic	15.35	30.25	1.953	101.8	316.3
Check-line	17.57	33.28	1.897	111.4	356.2
80-85 °C:					
High oleic	23.60	36.66	1.570	129.6	464.8
Check-line	23.85	38.19	1.598	134.7	469.3
85-90 °C:					
High oleic	24.96	38.83	1.558	136.5	489.3
Check-line	22.51	34.96	1.561	129.5	445.1
90-95 °C:					
High oleic	25.71	39.53	1.540	1.45.4	502.7
Check-line	23.66	36.95	1.564	126.8	465.9
100-105 °C:					
High oleic	24.03	39.07	1.628	135.0	472.5
Check-line	22.40	35.89	1.604	122.4	443.3

The results show that birds fed the 1.2% lysine diets (commercial soybean meal) performed significantly better in terms of their daily weight gain, feed conversion (feed:gain) and final body weight when compared to the test diets. This result is most likely attributable to the lower amino acid content of the test diets, although may also be due to differences in processing.

No significant differences in performance, in either the daily weight gain or the feed conversion, between the parental soybean line and the high oleic acid soybean line were observed.

Conclusion

Interpretation of both feeding studies is complicated by the fact that they were designed to look at the effect of a number of different parameters, other than soybean variety, on feeding performance (e.g., lysine content, processing temperature). Nevertheless, both demonstrate that the high oleic acid soybeans are equivalent to the commercial varieties of soybean in their ability to support typical growth and well-being in pigs and chickens.

5.3 Other relevant data

Studies submitted by Optimum Quality Grains:

The effect of using high oleic acid soybean oil to replace frying fats in targeted foods on the fatty acid composition of the diets of British adults. 1998. Study undertaken by Nutriscan Ltd, a non-profit making campus company of Trinity College, Dublin, Ireland.

To assess the nutritional impact of high oleic acid soybean oil the applicant commissioned a study on the effect of high oleic acid soybean oil on the balance of dietary fats in the human diet using dietary and nutritional survey data for British adults.

The fatty acid composition of high oleic soybean oil was compared with those of commercial shortenings and frying oils sourced from Europe and the United States. The key findings of these comparisons are:

- the level of saturated fatty acids in high oleic soybean oil is similar to that in non-hydrogenated or lightly hydrogenated oils and is considerably lower than most European shortenings;
- compared with frying oils with comparable levels of monounsaturated fatty acids, high oleic soybean oil has higher levels of n-6 polyunsaturated fatty acids (primarily linoleic acid);
- high oleic soybean oil is comparable with other frying oils for n-3 polyunsaturated fatty acids (primarily linolenic acid);
- high oleic soybean oil does not contain any of the trans isomers of unsaturated fatty acids found in many commercial shortenings.

For the dietary analysis two scenarios were modelled on the assumption that high oleic soybean oil replaced all oils present in savoury snacks, fried potatoes including chips and vegetables. It also assumed that frying oil accounted for 17% of the fat in all fried meat, eggs and fish. Because the composition of endogenous fat in the fried animal foods was not known, it had to be estimated for each food by difference between total fatty acids and a frying oil of known composition. In scenario I, a worst-case scenario, all the oil used for frying meat, eggs and fish was assumed to be a high n-6 polyunsaturated fatty acid (52.8%) corn oil. In scenario II, a more realistic scenario, the oil was assumed to be a palmolein/rapeseed (80:20) blend (12.3 % n-6 polyunsaturated fatty acids). Assumptions also had to be made about the level of n-6 polyunsaturated fatty acids in high oleic soybean oil as this level can be influenced by crop growth conditions. Commercially available high oleic soybean oil is anticipated to contain 2.2% n-6 polyunsaturated fatty acids but batches as low as 0.9% have been observed under certain field conditions. A n-6 polyunsaturated fatty acid content of 0.9% for high oleic soybean oil was assumed for scenario I and 2.2% was assumed for scenario II.

A summary of the main findings of the analysis is presented in Table 15 below.

Table 15: The effect of replacing all oils and fats used in the domestic and commercial frying with high oleic soybean oil (values are means ± standard deviations)

		High oleic soybean oil usage	
% energy from:	**Current diet[1]**	**Scenario I**	**Scenario II**
Saturated fatty acids	17.24 ± 3.44	16.61 ± 3.44	16.43 ± 3.43
Monounsaturated fatty acids	12.63 ± 2.15	14.97 ± 2.98	14.68 ± 2.86
n-3 polyunsaturated fatty acids	0.78 ± 0.27	0.73 ± 0.23	0.78 ± 0.23
n-6 polyunsaturated fatty acids	5.51 ± 2.15	3.89 ± 1.98	4.33 ± 1.92
Trans unsaturated fatty acids	2.24 ± 0.83	2.15 ± 0.83	2.12 ± 0.83

[1] no high oleic soybean oil usage

The analysis shows that the impact of the high oleic acid soybean oil use on the intakes of saturated fatty acids is quite small, equivalent to a 5% reduction at best, with little difference between the two scenarios. The intake of monounsaturated fatty acids would increase at best by 19%, with again little difference between the two scenarios. The intake of n-6

polyunsaturated fatty acids would fall by 29% for scenario I and by 21% for scenario II. The analysis also shows that there would be little or no change to the intakes of n-3 polyunsaturated fatty acids or trans unsaturated fatty acids with either scenario.

To put the use of high oleic soybean oil into context, the analysis was repeated using a low n-6 olive oil (79.3% monounsaturated fatty acids, 0.7% n-3 polyunsaturated fatty acids and 6% n-6 polyunsaturated fatty acids) to replace all of the fats and oils considered in the analysis. The results of this analysis are presented in Table 16 below.

Table 16: A comparison of the effect of replacing all oils and fats used in frying and in the manufacture of savoury snacks with either high oleic soybean oil or olive oil (values are means)

		% energy from			
Oil	**Scenario**	**Mono**	**n-6 poly**	**n-3 poly**	**Saturated**
High oleic	I	15.7	3.2	0.8	16.6
Olive	I	15.6	3.3	0.7	16.7
High oleic	II	15.1	4.2	0.8	16.1
Olive	II	15.0	4.3	0.8	16.2
CURRENT UK DIET		12.6	5.5	0.8	17.2

This analysis shows that, were low n-6 olive oil to replace all the fats considered in the analysis, the impact would be very similar to that of high oleic soybean oil under similar conditions.

The study concluded that while the use of high oleic soybean oil might lower dietary linoleic acid intake somewhat (by an absolute maximum of 29%), it would not do so to any level that would be a public health concern in terms of cardiovascular disease. Moreover, it was concluded that such a reduction could apply equally to many existing commercially available low n-6 polyunsaturated frying oils, such as olive oil.

Therefore, the overall finding of the study was that the nutritional impact of the use of high oleic acid soybean oil as a replacement for frying fats was likely to be beneficial because diets incorporating high oleic acid soybean oil show decreased saturated fatty acid intakes and this is likely to reduce risk factors for cardiovascular disease.

The general conclusion of this report can be applied to the Australian context although the magnitude of the changes are likely to be reduced. Table 17 shows a comparison of the fatty acid profiles of the United Kingdom and Australia from recent national dietary surveys.

Table 17: A comparison of mean percentage energy from fatty acids in British and Australian diets

Country	**Mean % En from fatty acid type**		
	Mono	**Poly**	**Saturated**
United Kingdom	12.6	6.3	17.2
Australia	11.8	5.0	12.7

The fall in mean polyunsaturated intakes quoted for the British case above assumes 100% replacement. In reality, this is unlikely to happen, and data given in the report show that, with successive reductions in the % replacement, intakes progressively increase towards original levels. For example at 25% percent replacement, percentage energy from PUFA decreases to 6.0%.

There are some high monounsaturated oils available or soon to be available on the Australian market that have been created through conventional plant breeding and selection techniques from sunflower and rapeseed stock. These types of oils have been successful in replacing a proportion of palm oil mixes in food manufacture and retail frying. Olive oil has also become a popular oil for domestic use.

Acknowledgements

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ATTACHMENT 3

DRAFT REGULATORY IMPACT ASSESSMENT

Regulatory Impact Assessment

The Authority is required, in the course of developing regulations suitable for adoption in Australia and New Zealand, to consider the impact of various options (including non-regulatory options) on all sectors of the community, including consumers, the food industry and governments in both countries. The regulatory impact assessment will identify and evaluate, though not be limited to, the costs and benefits of the regulation, and its health, economic and social impacts.

Identification of affected parties

1. Governments in Australia and New Zealand

2. Consumers in Australia and New Zealand

3. Manufacturers, producers and importers of food products

Options

Option 1–To prohibit the sale of food produced using gene technology

GOVERNMENT Commonwealth, New Zealand Health Departments, State/Territory Health Departments	**Benefits** • no benefits were identified.	**Costs** • the governments of Australia and New Zealand may be challenged under the WTO to justify the need for more stringent restrictions than apply internationally. • a prohibition on food produced using gene technology in Australia and New Zealand could result in retaliatory trade measures from other countries. • there may be technical problems for AQIS in enforcing such a prohibition at the import barrier.
INDUSTRY Manufacturers, producers and importers of food products	**Benefits** • Some companies may benefit from being able to exploit niche markets for non-GM products overseas.	**Costs** • food manufacturers and producers will be unable to use the processed food fractions from foods produced using gene technology thus requiring the switch to non-GM ingredients and the reformulation of many processed food products. The cost to manufacturers of going non-GM has been estimated to be $A 207m in Australia and $NZ 37m in New Zealand[5]. This is equivalent to 0.51% of turnover in Australia and 0.19% in New Zealand.

[5] Report on the cost of labelling genetically modified foods (2000)

CONSUMERS	**Benefits** • no benefits were identified, however as some consumers perceive GM food to be unsafe, they may perceive prohibition of GM food to provide a public health and safety benefit.	**Costs** • could lead to decreased availability of certain food products. • increased costs to consumers because manufacturers and producers may have to source non-GM ingredients.

Option 2– to permit the sale of food produced using gene technology

GOVERNMENT Commonwealth, New Zealand Health Departments, State/Territory Health Departments	**Benefits** • increased innovation and competitiveness in the food industry will benefit the economy.	**Costs** • minor costs associated with amending the *Food Standards Code.*
INDUSTRY Manufacturers, producers and importers of food products	**Benefits** • food producers and manufacturers will be able to capitalise on the latest technology. • food importers will continue to be able to import manufactured products from overseas markets including the USA and Canada where there is no restriction on the use of food produced using gene technology.	**Costs** • there may be some discrimination against Australian and New Zealand food products in overseas markets that have a preference for non-GM foods (e.g., Japan and the European Union).
CONSUMERS	**Benefits** • consumers may have access to a greater range of food products.	**Costs** • those consumers who wish to avoid GM food may experience restricted choice in food products. • those consumers who wish to avoid GM food may have to pay more for non-GM food.

Conclusion of the regulatory impact assessment

Consideration of the regulatory impact for foods produced using gene technology concludes that the benefits of permitting foods produced using gene technology primarily accrue to the government and the food industry, with potentially a small benefit to consumers. These benefits are considered to outweigh the costs to government, consumers and industry, provided the safety assessment does not identify any public health and safety concerns.

ATTACHMENT 4

WORLD TRADE ORGANISATION AGREEMENTS

With the completion of the Uruguay Round of trade negotiations, the World Trade Organisation (WTO) was created on 1 January 1995 to provide a forum for facilating international trade.

The WTO does not engage in any standard-setting activities but is concerned with ensuring that standards and procedures for assessment of and conformity with standards do not create unnecessary obstacles to international trade.

Two agreements which comprise part of the WTO treaty are particularly important for trade in food. They are the:

- Agreement on the Application of Sanitary and Phytosanitary Measures (SPS); and
- Agreement on Technical Barriers to Trade (TBT).

These agreement strongly encourage the use, where appropriate, of international standards, guidelines and recommendations, such as those established by Codex (in relation to composition, labelling, food additives, veterinary drug and pesticide residues, contaminants, methods of analysis and sampling) and the code and guidelines on hygienic practice.

Both Australia and New Zealand are members of the World Trade Organization (WTO) and signatories to the agreements on the Application of Sanitary and Phytosanitary Measures (SPS agreement) and on Technical Barriers to Trade (TBT agreement). Within Australia, a memorandum of understanding binding all States and Territories to the agreements has been put in place by the Council of Australian Governments (COAG).

The WTO agreements are predicated on a set of underlying principles that standards and other regulatory measures should be:

- based on sound scientific principles;
- developed using consistent risk assessment practices;
- transparent;
- no more trade-restrictive than necessary to achieve a legitimate objective;
- recognise the equivalence of similar measures in other countries; and
- not used as arbitrary barriers to trade.

As members of the WTO both Australia and New Zealand have an obligation to notify the WTO of changes to food standards to enable other member countries of the WTO to make comment. Notification is required in the case of any new or changed standards which may have a significant trade effect and which depart from the relevant international standard (or where no international standard exists). Matters raised in this proposal may be notified to the WTO as either SPS notifications or TBT notifications, or both.

SPS Notifications

These are primarily health related, and refer to any measure applied:

- to protect animal or plant life from risks arising from the entry, establishment or spread of pests, diseases or disease carrying organisms;

- to protect human or animal life or health from risks arising from additives, contaminants, toxins or disease-carrying organisms in foods, beverages or foodstuffs;

- to protect human life or health from risks arising from diseases carried by animals, plants or products thereof, or from the entry, establishment or spread of pests; and

- to prevent or limit other damage from the entry, establishment or spread of pests.

The Agreement on the Application of Sanitary or Phytosanitary Measures relates to any measure applied to protect animal, plant or human life or health which may directly or indirectly affect international trade. Whether the SPS measure is in the form of a law or mandatory regulation, an advisory guideline, a code of practice or a requirement, it is the purpose of the measure that is important - not its regulatory status. Each WTO member country is entitled to apply SPS measures that are more stringent than the international standards in order to protect the health of its population. In the interests of transparency, each instance of such non-alignment which could result in an impediment to trade must be identified and justified and the documentation of that justification must be readily available

Each member country is also required to apply its methods of risk assessment and management consistently so arrangements under the SPS Agreement do not generate what may really be technical barriers to trade

Under the SPS Agreement, an exporting country can have resort to the WTO's dispute settlement procedures with respect to such a non-alignment. These arrangements mean there is potential for a code of practice to introduce an SPS measure that may bring about non-alignment with international requirements. Such non-alignment would need to be justified scientifically on the grounds that it is necessary to protect human, animal or plant life or health.

TBT Notifications

A technical barrier to trade arises when a mandatory requirement in a country's food regulatory system does not align with the international standard and it is more trade restrictive than is necessary to fulfil a legitimate objective. However, it can be acceptable for a country to have a more stringent requirement than that set internationally for reasons including:

- Maintaining national security;
- Preventing deceptive practices; and
- Protecting human health or safety.

Instances of non-alignment with international standards which could result in trade barriers must be identified and, if questioned, justified. Voluntary codes of practice are not expected

to generate technical barriers to trade except where compliance with a code of practice or some aspect of a code of practice is expected. Consequently, it is possible for a voluntary code of practice to be viewed by the WTO as mandatory and subject to all the notification and other provisions applying to mandatory regulations.

The Agreement on Technical Barrier to Trade relates to requirements covering product characteristics or their related processes and production methods. TBT covers measures that are not SPS, such as requirements relating to terminology, symbols, packaging, marking, labelling, food composition and processing methods.

It is considered that these Full Assessments do constitute a potential Technical Barrier to Trade or a Sanitary/Phytosanitary matter. Matters raised in these Full Assessments therefore will be notified to the WTO.

ATTACHMENT 5

SUMMARY OF FIRST ROUND PUBLIC SUBMISSIONS FOR APPLICATIONS A372, A375, A378, A379, A380, A381, A382, A383, A384, A385, A386, A387 & A388

1. National Genetic Awareness Alliance (Aus)

- Believes that the patenting of life-forms and living processes represents a violation of human rights, threat to food security, impediment to medical research and a threat to animal welfare
- Believes that current GM techniques are inherently hazardous, and have been shown recently to offer no benefits
    - Lower yields with high pesticide input
    - Intensification of the corporate monopoly on food
    - Spread of antibiotic resistance marker genes and promoter sequences
    - Possible increase of allergenicity due to spread of transgenic pollen
- Urges governments to use precautionary principle and carry out research into sustainable agricultural methods
- Calls for suspension of trials and sale of GM products and public inquiry.

2. Pola Lekstan and Anna Clements (Aus)

- Are concerned that approval without long-term testing may pose a health threat, that more GM food means less choice for those wanting to avoid it, that Bt may affect non-target organisms, and that herbicide resistance may lead to overuse of chemicals.

3. Arnold Ward (Aus)

- Questions the system of MRL setting in light of the levels of high glyphosate residues in Roundup Ready soybeans and of other chemicals (including the Bt toxin) in GM crops
- Is concerned about detrimental effect of Bt on non-target (beneficial) organisms and on humans, and believes that genetic engineering is imprecise with uncertainties in outcomes
- Believes that the concept of substantial equivalence is inadequate and should not be used to avoid more rigorous testing, and that commercial factors are overriding need for basic research. Also believes that ANZFA's arguments defend the needs of biotechnology companies and food processing industry, and that since ANZFA does no testing itself, the results can't be trusted.

4. Australian GeneEthics Network

- Believes that the data provided is insufficient to make an assessment, and clock should be stopped on the applications. Concerns include:
    - Direct health effects of pesticide residues
    - Possibility of transfer of antibiotic resistance marker genes leading to resistant bacteria
    - The possibility that transfer of other traits e.g. herbicide tolerance to bacteria, could lead to horizontal spread of unfavourable traits
    - Insertion of viral DNA could create new and virulent viruses
    - The possibility that approval could lead to the growing of GMOs in Australia – ecological concerns including effects of, and increases in resistance to, Bt-toxins and the encouragement of increased herbicide use resulting from herbicide-tolerant crops
    - The threat to GE-free status export markets
- Believes that the term 'substantial equivalence' is not useful– compositional data alone does not establish equivalence

5. Public and Environmental Health Service (Aus)

- Believes that the data provided should cover both the intentional and unintentional effects of the genetic modification. The unintended consequences of random insertion of new genetic material into the host genome could include loss or change of function of gene or controlling element, disregulation or amended regulation of the gene or controlling element, or production of a novel hybrid protein which could occur in an unregulated manner. They should also cover any compositional changes e.g. nutrients, antinutritional factors, natural toxicants, and define when a change would be considered 'significant'
- Potential effect of introduced proteins on metabolic pathways should be addressed e.g. over-expression or inhibition of enzymes
- Data should include details of whether introduced proteins are detectable in whole commodities, processed products and highly processed derivatives
- Data should include details of toxicity and allergenicity tests to prove that food is safe, as well as address issues of specificity and potency of proteins. It should also address the ability to support typical growth and well-being
- Data for herbicide-tolerant plants should be derived from studies performed on plants treated with herbicide. They should address the human toxicity of the herbicide and whether residues of the herbicide degradation process are present, toxic and/or subject to an MRL.

6. David Grundy (Aus)

- Considers that the expression of Bt toxins and other chemicals in plant tissues removes the choice of washing chemicals off fruit and vegetables. Believes that Roundup Ready crops have glyphosate or glufosinate molecules genetically attached
- Believes that GM crops should not be used for feed given to animals bound for human consumption, that products encouraging antibiotic resistance should not be used, and that labelling should be mandatory for all products containing GM ingredients

7. Leesa Daniels (Aus) Member of the Genetic Engineering Action Group

- Believes that:
    - Scientific research although limited, has brought concerns to light
    - Substantial equivalence is a subjective principal
    - Comprehensive and mandatory labelling must be urgently implemented
    - The Cauliflower Mosaic Virus (CaMV) promoter could enhance the capability to transfer genes horizontally and has the potential for activating dormant or new viruses
    - Antibiotic marker genes could lead to increase in antibiotic resistance
    - Several of the transformations encourage the use of pesticides, all of which have shown to be harmful.

8. Australian Food and Grocery Council

- Fully endorses the policy of minimum affective regulation, supports these applications, and considers that food manufacturers should make their own choice with regard to use of GM crops or products derived from them
- Believes that since the growth of GM crops has been approved overseas, they would support their growth in Australia if approved through the GTAC/GMAC/OGTR process
- Considers it unfortunate that ANZFA has not negotiated "equivalence" agreements for products already approved overseas to enable approval without having to carry out its own safety assessment. In the absence of such an agreement it supports the ANZFA safety assessment process.

- Believes that an appropriate information and labelling scheme would enable consumers to make an informed choice

9. New Zealand Ministry of Health

- Referred preliminary report to New Zealand Health Research Council, who stated concern that all safety aspects should be carefully considered in the ANZFA process.

10. Nestle Australia Ltd.

- Supports the continued approval of glufosinate ammonium-tolerant canola, and believes that manufacturers would be disadvantaged were approval not to be granted.

11. Consumers' Association of South Australia Inc. & National Council of Women of Australia (CASA supports submission of NCWA)

- Believe that current testing procedure is inadequate and that human trials are the only adequate method, as with testing of new drugs. Also that physiological and neurological effects as well as the toxicological and allergenic effects should be looked at, and that an independent body should be responsible for testing
- Do not support the use of antibiotic markers, since they believe they may pose a threat to efficacy of antibiotics in humans
- State that new research has shown that GM soybeans may be a less potent source of phytoestrogens than conventional soybeans confirming the inadequacy of the term 'substantial equivalence'
- Raise the point that although these crops have been approved elsewhere, this situation may change with consumer pressure
- Do not accept that it is impossible to source food to ascertain whether or not it contains GM ingredients. Believe that if McCain and Sanitarium can do it, then others should also be able to
- State general concern about the risk that MRLs will be raised as a result of herbicide-tolerant crops being developed, and feel that the calculations used are flawed and are not based on safety criteria
- Believe that the use of GM crops in animal feed should also be regulated. A378
- State concern over possible increase in glyphosate use (it is apparently confirmed in one reference that herbicide use increases with herbicide resistant crops), referring to studies that link the chemical to Hodgkin's lymphoma, and the possibility that Europe may ban it due to adverse effects on beneficial insects**.** They are particularly concerned that glyphosate is not looked at by the same regulatory body as that looking at GM foods

A379, A388

- State concern over the persistence and toxicity of bromoxynil, and consider that these have not been adequately assessed by the US FDA. They understand that the breakdown product of bromoxynil (DBHA) may be more potent than bromoxynil itself, and believe that a safety assessment needs to be done on this too. This is apparently the main residue, and they believe that this may appear in cotton oil and linters.

A372, A375, A380, A381, A386

- With respect to glufosinate ammonium, state concern about toxicity, neurotoxicity, teratogenicity and residues in food, soil and water. They believe that Monsanto is likely to apply for an increase in the MRL, and that such increases are likely to constitute a health hazard

A380, A382, A383, A384, A385, A386

- Raise issues of adverse effects of Bt toxins on non-target insects and think that it needs more study.

A387

- Believe that raising the amount of a nutrient in a food may have unknown drawbacks e.g. affecting the efficacy of other nutrients

12. Health Department of Western Australia

- Highlights various health and environmental concerns:
    - the use of antibiotic resistance genes as markers may transfer resistance to animals via gut bacteria
    - the possibility that microbial gene sequences may contain fragments of other virulent genes, and also that ingesting Bt toxins may be harmful to humans
    - the possibility that insects may be more prone to developing resistance to Bt, since Bt toxins have been found to be released into the soil
- Believes that both safety data and gene sequences should be available for public scrutiny

13. Meat New Zealand

A379

- Concerned at how labelling regulations will apply to sausage casings that may contain cotton linters even if they are not to be eaten, i.e. are effectively a processing aid. Think that labelling should only be used to advise the sausage manufacturer not consumers.

14. BRI Australia

- Supports the approval of all 13 applications provided ANZFA is satisfied with their safety

15. Food Technology Association of Victoria Inc.

- Supports the approval of all 13 applications provided ANZFA is satisfied with their safety

16. Diane Davie (Aus)

- Believes all 13 applications should be rejected, since they have not undergone human safety testing here or overseas, and have not been assessed on their ethical merits
- Believes that risks include:
    - Bacterial and viral vectors which could affect human physiology
    - Herbicide and insect-resistance genes, which could increase allergies and antibiotic resistance
    - Environmental risks
- Also believes that ANZFA must heed the concerns of consumers opposed to GM foods

17. Martin Hurley, David Hook, Ian Smillie, Margaret Dawson, Tee Rodgers-Hayden, David Lovell-Smith (Natural Law Party), Barbara Brown, Ngaire Mason, Robert Anderson (member, Physicians and Scientists for Responsible Genetics), Louise Carroll, Gilbert Urquart, Caroline Allinson-Dunn, Megan Lewis, Peter Barnes, James Harlow, Gabrielle Dewan, Scott Young, Virginia Murray, Stephanie Chambers, Kay Dyson, Peter Fenwick, Joanne Xerri, Paul True, Josh Gill, James & Peysha Charlwood, Mitta Hirsch, Alan Florence, Nicole Paul, Lawrence Clarke, David Snowman, Reg Paling, Mark and Johanna Blows, David and Bev Semour, Richard and Sharon Moreham (see also below), Stuart Drury and Helen Murphy (All Aus), Brennan Henderson (NZ) – Generic e-mail objection

- Believe that most Australians and New Zealanders do not want GM foods, there are no benefits, and deferral would not be disadvantageous. Approval should be delayed until they are proven safe.

- Feel that there is insufficient time to assess these applications thoroughly, and there are so many products under development that there is a high overall risk of a major disaster
- Believe that GM foods encourage pesticide use, and applications have made for commercial purposes only, and also that here could be commercial benefit to Australia and New Zealand in remaining GM-free.

18. Richard and Sharon Moreham (see also above)

- In addition to the points above, also think that it is unfortunate that the NZ government agreed to joint approval of food, as the Australian public are less educated about the issues surrounding GM foods
- Think that approval would only prove that ANZFA serves the interests of large multinational companies rather than those of the public.

19. Vicky Solah (Aus)

- Is for GM foods if the safety evaluation is carry out using approved, validated methods by an independent body, if the results are made available to consumers, and if all GM food is labelled
- Is concerned that transformation may lead to disruption of another gene, and that more research is needed before it is clear whether the process is safe
- With regard to herbicide tolerant crops, is concerned that consumers may not be aware of the need to wash products that have been sprayed, and that this therefore impacts on food safety. Also concerned about environmental impact of these chemicals, and of the possibility of resistance necessitating higher pesticide use in the future.

20. Dr Rosemary Keighley (Aus)

- Will not purchase foods unless they are certified GM-free. Believes that Australian producers who do not actually use GM products, but who fail to label them as such, will suffer.

21. Nicola Roil (Aus)

- Believes that GM foods pose health threats and may contaminate non-modified crops

22. Ian and Fran Fergusson (Aus) – also wrote in the big lot above

- Believe there has been inadequate testing, and are concerned about possible side-effects

23. Lyndal Vincent (Aus)

- Urges delay of approval until proven safe by extensive testing. Considers that genetic material is being released without knowing what the effects are, and cannot be recalled.
- Believes that there is no benefit to the consumer, and that national economic interests are best served by maintaining a GM-free market.

24. Fay Andary (Aus)

- Does not want any of the 13 products covered by the applications to be approved for inclusion in the food supply

25. John and Francesca Irving (Aus)

- Thinks that no GE foods should be approved for inclusion in the food chain

26. Diana Killen (Aus)

- Believes that there is no proven benefit to consumers and in many instances nutritional value is actually lower in GM crops, and it is therefore irresponsible to push through approval without thorough assessment of their long-term safety for public health.
- Suggests that research has highlighted adverse allergic reactions and a lowered immune response in some individuals, and that there are health implications with crops designed to be grown with greater concentrations of pesticides

- Thinks that labelling is essential for consumers to discriminate in purchasing, and that Australia has a unique opportunity in supply of organic and GM-free food.

27. Sheila Annesley (Aus)

- Does not want any of the 13 foods included in the food supply.

28. David and Edwina Ross (Aus)

- State concern for the future food supplies and well-being of their grandchildren.

29. Beth Schurr (Aus)

- Wishes to protest against the threat of GM foods, the possible future detrimental effects and the further endangering of the planet.

30. Beth Eager (Aus)

- As a parent is concerned that neither the long-term effects on health nor the environment are being considered.

31. Bruce Pont and Ljiljiana Kuzic-Pont (Aus)

- Believe that safety has not been, and cannot be satisfactorily determined, and that any party associated with GM foods could be legally liable should adverse health effects be seen. Thalidomide, smoking, 'Agent Orange' and asbestos all show that such things can affect subsequent generations
- Believe that an increase in use of pesticides will result from pesticide-tolerant crops, and that the emphasis should be on organic and/or safe agriculture
- Believe that GM-food is a retrograde step, contrary to nature and has the potential to destroy the human race.

32. Chitta Mylvaganum (Aus)

- Wishes to know what tests were done to assess negative effects on human and environmental health, how thorough they were, what the outcomes were, are the results publicly available, and what further avenues of inquiry are open to the public
- Requests the prevention of the import or release of any products until tests are carried out by unbiased scientists in order to prove the lack of health or environmental effects.

33. John Stevens (Aus)

- Would be concerned if approval were granted before sufficient research had been completed on potential impacts on human health and gene pools of nearby crops. Once grown, spread via pollen would be impossible to stop, and labelling would not prevent exposure by this route
- Considers that utmost caution should be exercised and import approval denied indefinitely

34. Tim Carr (Convenor of the Emergency Committee against GE Foods)

- Believes that GM-foods are produced using a radical and unpredictable new technology so should be subject to more rigorous testing
- States that it is unknown how the introduced gene will interact with and influence genetic expression in the host genome, and could change the chemical nature of the food
- Considers that health risks could result from the increased use of pesticides, and also that ANZFA should consider wider environmental, ethical and socio-economic issues.

35. Jan Kingsbury (Aus)

- Believes that GM-foods could result in loss of economic advantage for Australia and New Zealand since they are known internationally for pure and safe products
- Believes that foods are being complicated and pushed by big internationals, and organic farmers are being contaminated by cross-pollination

36. Teresa Sackett (Aus)

- Believes that:

- The KPMG report on labelling was prepared in a ridiculously short time and provided limited analysis
- The proposal of 'no label' for foods which 'may contain' or in which there is 'no evidence' of GM material is inadequate
- Inadequate testing procedures should not be used to declare a product is GM-free just because material can't be detected. In fact testing methods have been developed that can be used to work out the GM content
- Government and industry seem to be favouring the introduction of GM foods. This will result in:
  - Increased use of chemicals
  - Destruction of soil life
- Organic farming pay high costs for producing healthy plants, while conventional farmers have little restriction on pollution of air, soil and water. Salinity problems, the death of the Great Barrier Reef, rivers and streams has resulted from ignorance in farming and broader community. Such problems will increase with GM foods.
- The implication that the public will not understand the issues is wrong. Everyone needs to be fully informed.

- Asks the question of whether workers in the food industry are to be better informed, and also why no 'verification documents' are to be required by retailers? Believes that certification schemes should be on a par with those for Kosher foods and organics

37. John and Sandy Price (Aus)

- Approval of GM foods and seeds should not be allowed, as it is an affront to the sovereignty of Australia and the dignity of the Australian people. The results of the experiment cannot be reversed.

38. John Scott (NZ)

- Encloses article from The Irish Times, which describes the restrictions that have been placed by the US EPA on the cultivation of GM corn. These appear to have resulted from fears that Bt crops may be harmful to Monarch butterflies and that resistance may develop to Bt

39. R A Randell (NZ)

- Believes that all GM products should be placed under a moratorium until the Royal Commission of Inquiry has considered the issue, and until all scientific, philosophical, ethical and moral issues have been looked at.

40. National Council of Women of New Zealand

- Believes that:
  - approval of all 13 applications should be rejected, and that none should be approved for planting.
  - Independently-funded body should be responsible for safety assessments
  - If it is possible to segregate high-oleic soybeans, then RoundUp Ready soybeans should be segregated too
  - Consumers should be made aware of the extent of GM ingredients in their food
  - GM foods, additives or processing aids already on the market must be labelled comprehensively and without extra cost to the consumer – suggest 'GM unknown' rather than 'may contain'
- Appreciates that rejection may contravene the WHO agreement, but consider that the primary role of ANZFA is the assurance of health and safety

41. Safe Food Campaign (NZ)

- Believes that approval should be rejected, and a moratorium be put in place until after the Royal Commission of Inquiry, for various reasons:

- Possible effects on non-target insects
- Spread of GM pollen may cause contamination of non-GM (especially organic) crops, and may result in the spread of herbicide-tolerance genes and an increase in resistance development. Cross-pollination is considered a particular risk for canola (A372 & A388). Bt resistance development is noted as being a particular risk for A382, A383 & A384
- Lack of long-term testing means health risks are not known
- Use of broad-spectrum pesticides affects wild flowers and non-target insects.

42. Jocelyn Logan, Caroline Phillips (NZ)

- Oppose all 13 applications for the following reasons:
  - Testing has not been long-term or independent, precautionary principle should apply. Approval can happen later if GM is proven safe.
  - No clear public benefit, and lack of opportunity for informed choice (immoral and undemocratic). Labelling regulations also unsatisfactory in this respect.
  - Environmental concerns (increase in pesticides, threat to organic farming, Bt resistance)

43. Robert Anderson (member of Physicians and Scientists for Responsible Genetics - NZ)

- Considers that the GM issue should be reconsidered in the light of the release of internal FDA documents made available for a recent lawsuit aimed at amending their policy. Attached document (presentation given by Steven Druker, Alliance for Bio-integrity) suggests that:
  - Scientist's warnings have been ignored
  - FDA policy may be illegal, violating the Food, Drugs and Cosmetic Act – Mr Druker believes that the term generally-regarded-as-safe (GRAS) cannot apply to foreign DNA

44. Stephen Blackheath (NZ)

- Argues that ANZFA's approach to safety assessments is scientifically unsound:
  - Antibiotic resistance marker genes have been cited as being potentially dangerous by groups other than ANZFA e.g. the Royal Society
  - Unanticipated toxins and allergens are a concern, and it is suggested that the ANZFA process does not adequately consider these possibilities
  - Doesn't address the question of whether risks exist that are unique to the GM process
  - It relies on data from the manufacturers themselves, with little sway given to evidence from public submissions. Companies have vested interests the results and cannot be trusted (also gives evidence of Monsanto's past dishonesty)
- Believes that ANZFA is subject to undue influence through the directors, and is biased towards being pro-GM
- Suggests that RoundUp Ready soybeans are not substantially equivalent as the stems have been found to be more brittle than traditional lines, and may be lower in phytoestrogen content
- Also cites the lawsuit being brought by the Alliance for Bio-integrity, and the internal FDA documents that suggest concern from FDA scientists, as evidence of the FDA ignoring important evidence.

45. Claire Bleakley (NZ)

- Believes that approval should be rejected for various reasons:
  - They may be against Maori views

- Further long-term trials are needed and should be carried out by ANZFA themselves - certain trials have apparently shown effects on immune system, allergies and rare syndromes
- Health concerns of pesticide overuse
- The possibility of horizontal gene transfer with respect to antibiotic resistance transfer
- Lack of labelling and the use of the unsatisfactory 'substantial equivalence' concept, which makes hazard difficult to assess
- There is no substantial gain to consumers

ATTACHMENT 6

GENERAL ISSUES RAISED IN PUBLIC COMMENTS

The majority of submissions received in response to the Section 14 Gazette Notice, expressed general views against the use of gene technology and asserted that food produced using this technology is unsafe for human. A number of general issues were raised in these submissions and are addressed below.

1. *The safety of genetically modified foods for human consumption*

A majority of submitters raised the issue of public health and safety in relation to food produced using gene technology. In particular, it was stated that there has been inadequate testing of genetically modified foods, that there is limited knowledge concerning the risks associated with the technology and that there may be potential long–term risks associated with the consumption of such foods.

- *Evaluation*

It is a reasonable expectation of the community that foods offered for sale are safe and wholesome. In this context, 'safe' means that there is a reasonable certainty of no harm. As with other aspects of human activity, the absolute safety of food consumption cannot be guaranteed. Conventionally produced foods, while having a long history of safe use, are associated with human disease and carry a level of risk which must be balanced against the health benefits of a nutritious and varied diet.

Because the use of gene technology in food production is relatively new, and a long history of safe use of these foods has yet to be established, it is appropriate that a cautious approach is taken to the introduction of these foods onto the market. The purpose of the pre–market assessment of a food produced using gene technology under Standard A18 is to establish that the new food is at least as safe as existing foods. The comprehensive nature of the scientific safety assessment, undertaken on a case-by-case basis, for each new modification is reflective of this cautious approach.

The safety assessment focuses on the new gene product(s), including intentional and unintentional effects of the genetic modification, its properties including potential allergenicity, toxicity, compositional differences in the food and it's history of use as a food or food product.

Foods produced using gene technology are assessed in part by a comparison with commonly consumed foods that are already regarded as safe. This concept has been adopted by both the World Health Organisation (WHO)/Food and Agriculture Organisation (FAO) and the Organisation for Economic Cooperation and Development (OECD). The Authority has developed detailed procedures for the safety assessment of foods produced using gene technology that are consistent with international protocols developed by these bodies.

2. *The need for long-term feeding studies*

A number of submissions were concerned about the lack of long-term toxicity studies on genetically modified foods.

- *Evaluation*

Animal studies are a major element in the safety assessment of many compounds, including pesticides, pharmaceuticals, industrial chemicals and food additives. In most cases, the test substance is well characterised, of known purity and of no nutritional value, and human exposure is generally low. It is therefore relatively straightforward to feed such compounds to animals at a range of doses (some several orders of magnitude 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.

By contrast, foods are complex mixtures of compounds characterised by wide variations in composition and nutritional value. Due to their bulk, they can usually be fed to animals only at low multiples of the amounts that might be present in the human diet. Therefore, in most cases, it is not possible to conduct dose-response experiments for foods in the same way that these experiments are conducted for chemicals. In addition, a key factor to be considered in conducting animal studies on foods is the need to maintain the nutritional value and balance of the diet. A diet that is poorly balanced will compromise the interpretation of any feeding study, since the effects observed will confound and usually override any small adverse effect which may be related to a component or components of the food. Identifying any potentially adverse effects and relating these to an individual component or characteristic of a food can, therefore, be extremely difficult. Another consideration in determining the need for animal studies is whether it is appropriate from an ethical standpoint to subject experimental animals to such a study if it is unlikely to produce meaningful information.

If there is a need to examine the safety of a newly-expressed protein in a genetically-modified food, it is more appropriate to examine the safety of this protein alone in an animal study rather than when it is part of a whole food. For newly-expressed proteins in genetically-modified foods, the acute toxicity is normally examined in experimental animals. In some case, studies up to 14 days have also been performed. These can provide additional re-assurance that the proteins will have no adverse effects in humans when consumed as part of a food. Such experiments can provide more meaningful information than experiments on the whole food. Additional re-assurance regarding the safety of newly-expressed protein can be obtained by examining the digestibility of the new protein in *in vitro* assays using conditions which simulate the human gastric system.

3. *Substantial equivalence*

A number of submitters expressed concern regarding the use of the concept of substantial equivalence as part of the assessment process. Some rejected the premise of substantial equivalence on the grounds that differences at the DNA level make foods substantially different.

- *Evaluation*

Substantial equivalence embodies the concept that, as part of the safety assessment of a genetically modified food, a comparison can be made in relation to the characteristics and properties between the new food and traditionally-produced food. This can include physical characteristics and compositional factors, as well as an examination of the levels of naturally occurring allergens, toxins and anti-nutrients.

This allows the safety assessment to focus on any significant differences between the genetically modified food and its conventionally produced counterpart. Genotypic differences (i.e. differences at the DNA level) are not normally considered in a determination of substantial equivalence, if that difference does not significantly change the characteristics for composition of the new food relative to the conventional food.

The concept of substantial equivalence allows for an evaluation of the important constituents of a new food in a systematic manner while, recognizing that there is general acceptance that normally consumed food produced by conventional methods is regarded by the community as safe. It is important to note that, although a genetically modified food may be found to be different in composition to the traditional food, this in itself does not necessarily mean that the food is unsafe or nutritionally inadequate. Each food needs to be evaluated on an individual basis with regard to the significance of any changes in relation to its composition or to its properties.

The concept of substantial equivalence was first espoused by a 1991 Joint Consultation of the Food and Agricultural Organisation (FAO) and the World Health Organisation (WHO) where it was noted that the '*comparison of a final product with one having an acceptable standard of safety provides an important element of safety assessment*.'
The concept has been internationally recognised and embraced as a valuable tool in the safety assessment of foods produced using gene technology. The OECD also advocates an approach to safety assessment based on substantial equivalence as being '*the most practical to address the safety of foods and food components derived through modern biotechnology*.'

4. *The nutritional value of food produced using gene technology*

A small number of submitters expressed concern that the genetic alteration of food decreases its nutritional value.

- *Evaluation*

The assessment of food produced using gene technology by ANZFA entails an exhaustive evaluation of analytical data on any intentional or unintentional compositional changes to the food. This assessment encompasses the major constituents of the food (fat, protein, carbohydrate, fibre, ash and moisture) as well as the key nutrients (amino acids, vitamins, fatty acids). There is no evidence to suggest that genetic modification *per se* reduces the nutritional value of food.

In the future, genetic modification may be used intentionally to improve the nutritional value of food. In this regard, GM foods may be able to assist in addressing the general nutritional needs of the community and also specific dietary needs of sub-populations.

5. *Potential toxins and allergens*

Some submitters expressed concerns about the risks of the introduction of new toxins or allergens.

- *Evaluation*

This issue is considered in detail as part of the safety assessment conducted on each new genetic modification applied to a food or commodity crop. New toxins or allergens may be introduced into food by either gene technology or by traditional breeding techniques, or by altered production processes. It is also possible to use these techniques to develop foods specifically where such compounds are significantly reduced or eliminated. One advantage of gene technology, in comparison with these other methods, is that any transferred genes are well characterised and defined, thus the possibility of developing a food with a new toxic or allergenic compound is likely to be reduced.

6. *Antibiotic resistance*

Some submitters raised concerns about increased antibiotic resistance resulting from the use of gene technology. Some felt that it would be reassuring if independent biomedical advice were available to reassure the public that the use of antibiotic resistance markers does not pose a risk to the future use of antibiotics in the management of human disease.

- *Evaluation*

The human health considerations in relation to the potential for the development of antibiotic resistance depend on the nature of the novel genes and must be assessed on a case-by case basis. This issue arises because of the use of antibiotic resistance marker genes in the generation of genetically modified plants. In some circumstances, antibiotic resistance genes are linked to the gene of interest, to enable the initial selection of the engineered cells in the laboratory. Those cells that contain the antibiotic resistance marker gene, and hence the gene of interest, will be able to grow in the presence of the antibiotic. Those cells that failed the transformation process are eliminated during the selection procedure.

Concern has arisen that ingestion of food containing copies of antibiotic resistance genes could facilitate the transfer of the gene to bacteria inhabiting the gut of animals and humans. It is argued that these genes may then be transferred to disease causing bacteria and that this would compromise the therapeutic use of these antibiotics.

In 1993, the World Health Organisation Food Safety Unit considered this issue at a Workshop on the health aspects of marker genes in genetically modified plants. It was concluded at that Workshop that the potential for such gene transfers is effectively zero, given the complexity of the steps required. Since this time, several separate expert panels (Report to the Nordic Council, Copenhagen 1996; Advisory Committee on Novel Foods and Processes, UK 1994, 1996; The Royal Society, UK 1998) and numerous scientific papers published in peer reviewed journals have also considered the available evidence on this issue. It is generally agreed that the presence and subsequent transfer of an intact functional gene from transgenic food to micro-organisms in the human intestine is an extremely unlikely event. Furthermore, if this were to occur, bacteria would not normally retain the resistance genes unless there was an environment for positive selection. The majority of these genes provide for resistance to

antibiotics whose use is confined to the laboratory and are not considered to be of major therapeutic use in humans.

Antibiotic resistant bacteria are naturally occurring, ubiquitous and normally inhabit the gut of animals and humans. There is a general consensus that the transfer of antibiotic resistance genes is much more likely to arise from this source and from associated medical practices, rather than from ingested genetically modified food. Even so, at the recent OECD Conference (GM Food Safety: Facts, Uncertainties, and Assessment) held in Edinburgh on 28 February – 1 March 2000, there was general consensus that the continued use of antibiotic marker genes in GM food crops is unnecessary given the existence of adequate alternatives, and should be phased out.

7. Transfer of novel genes

Some submitters have expressed concern that the transfer of any novel gene may be a health concern.

- *Evaluation*

It is extremely unlikely that novel genetic material will transfer from GM foods to bacteria in the human digestive tract because of the number of complex and unlikely steps that would need to take place consecutively. It is equally unlikely that novel genetic material will transfer from GM foods to human cells via the digestive tract. In considering the potential impact on human health, it is important to note that humans have always consumed large amounts of DNA as a normal component of food and there is no evidence that this consumption has had any adverse effect on human health. Furthermore, current scientific knowledge has not revealed any DNA sequences from ingested foods that have been incorporated into human DNA. Novel DNA sequences in GM foods comprise only a minute fraction of the total DNA in the food (generally less than 0.01%) and are therefore unlikely to pose any special additional risks compared with the large amount of DNA naturally present in all foods.

8. *Viral recombination*

Some submitters expressed concern about the long term effects of transferring viral sequences to plants.

- *Evaluation*

This is an issue that is commonly raised because some of the genes that are transferred to plants use a plant virus promoter. Promoters are controlling DNA sequences which act like a switch and enable the transferred genes to be expressed (i.e. to give rise to a protein product) in a plant cell. The routine use of these viral promoters is often confused with research which has shown that plant virus genes, which have been transferred into plants to render them virus–resistant, may recombine with related plant viruses that subsequently infect the plant, creating new viral variants. This research demonstrates that there may be a greater risk to the environment if viral genes are transferred to plants because it may lead to the generation of new plant virus variants capable of infecting a broader range of plants. This is a matter that will be addressed by the Genetic Manipulation Advisory Committee (GMAC) on a case–by–case basis when it assesses such plants.

However, the presence of plant viruses, plant virus genes or plant virus segments in food is not considered to pose any greater risk to human health as plant viruses are ubiquitous in nature and are commonly found in food eaten by animals and humans. Plant viruses are also biologically incapable of naturally infecting human or animal cells.

9. *Labelling of foods produced using gene technology*

A majority of submissions focussed on this issue. Specifically, the submissions called for the labelling of all foods produced using gene technology, regardless of whether they are substantially equivalent to conventional foods. The submitters based their demands for full labelling on the presumption that all foods produced using gene technology are unsafe and on consumer "right to know" arguments. It was stated that full labelling was the only means of identification of foods produced using gene technology available to consumers.

- *Evaluation*

The existing Standard A18 already makes provision for mandatory labelling of genetically modified foods that are substantially different from their conventional counterparts. However, ANZFA is committed to implementing the in-principle decision of ANZFSC Health Ministers of August 1999 to require labelling of all genetically modified foods, including those that are substantially equivalent in composition to the unmodified form. In conjunction with a task force of officials from State and Territory Health Departments and the New Zealand Ministry of Health, ANZFA developed draft revision to Standard A18 in October 1999 that requires labelling of other categories of genetically modified foods. At the Ministers request this draft was circulated for public review and a cost-benefit analysis of full labelling was commissioned. The task force considered both public comments and the cost-benefit analysis in finalising their recommendations to Ministers, which were delivered in May 2000. Ministers are to meet to resolve the issue in July 2000 following whole-of-government consideration of the issue. It is therefore expected that, following a decision and legal amendments to the standard, labelling requirements will be implemented that will apply to all current and subsequent applications.

10. The need for post marketing surveillance of genetically modified foods

A number of submitters have commented on the need for post-market surveillance of genetically modified food consumption.

- *Evaluation*

Surveillance of potential adverse or beneficial effects of GM foods is seen by many as a logical follow-up to the initial scientific risk assessment. Nevertheless, it is recognised that there are limitations to the application of epidemiology studies, particularly in relation to food components. A key requirement for post-market surveillance systems is that a clear hypothesis be identified for testing. Establishing a system for the surveillance of potential health effects of exposure to novel foods requires monitoring of the consumption patterns of novel foods in the population, and health effects in both "exposed" and "non-exposed" individuals/populations, so that risk estimates can be derived. For any such monitoring system to be useful, there needs to be a range of exposures, otherwise, any variation in health

outcome would be unexplainable by that exposure. Variations in exposure could be apparent over time (temporal trends), space (geographical trends) or both.

Availability of robust data on consumption of the foods in question is vital in order to establish a surveillance system. The other side of the equation is the need for access to data on population health outcomes. Such a system could also be used to identify potential positive health outcomes, such as improved nutritional status or lower cholesterol levels. The availability of linked basic data (e.g. date of birth, sex, geographical location), and the ability to correlate with demographic data, could potentially offer the means of establishing links with food consumption.

The possibility of setting up a post-market health surveillance system for novel foods, including GM foods, has been examined by the UK's Advisory Committee on Novel Foods and Processes (ACNFP). Recognising the many difficulties involved in developing such a system, an initial feasibility study to look at the available data and its usefulness has been proposed. Work is currently being commissioned; when completed in 18 months, it will be subject to peer review. If such a feasibility study suggests that post-market surveillance is practical, methods and details concerning data collection will be determined in the UK, but common strategies might be able to be harmonised internationally in order to minimise the use of resources while maximising the reliability of the final results. This is an area that ANZFA will be monitoring closely, along with international regulatory bodies such as the OECD Taskforce for the Safety of Novel Foods and Feeds.

11. *Public consultation and information about gene technology*

A number of submitters were concerned that the public has not been properly consulted or informed by government or ANZFA on the introduction of foods produced using gene technology. Some submitters urged to undertake wider consultation with all affected parties including growers, the food industry and consumers before these food commodities are introduced, and to ensure that adequate consultation is undertaken as part of its assessment process.

- *Evaluation*

The issue of gene technology and its use in food has been under consideration in Australia since 1992. The Agreement between the Governments of Australia and New Zealand for a joint food standard setting system, however, did not occur until 1995, and the New Zealand community therefore had not been consulted on this matter by the Authority until after that time. Consequently, the proposed standard (the current Standard A18) underwent only one round of public comment in New Zealand at which time significant objections were raised by the New Zealand community to the use of gene technology in food production. Many New Zealand consumers, both in these submissions, and in previous submissions to the Authority, have expressed the view that there has been insufficient consultation and a consistent lack of information about gene technology.

Although Standard A18 came into force in May 1999, the public have a continuous and ongoing opportunity to provide comment in relation to applications under the standard. ANZFA's statutory process for all applications to amend the *Food Standards Code* normally involves two rounds of public comment. Furthermore, all the documentation (except for commercial in confidence information) relating to these applications is available in the public

domain, including the safety assessment reports. There is ample evidence that the provision of such information by ANZFA has already significantly stimulated public debate on this matter.

In addition, other government departments including the Environmental Risk Management Authority (ERMA), are potential sources of information about gene technology available to consumers in New Zealand. ERMA is a statutory authority set up by the New Zealand Government to administer the *Hazardous Substances and New Organisms (HSNO) Act 1996*, and has responsibility for assessing the risks to the environment from genetically modified organisms. This body has been assessing applications for the approval of genetically modified organisms since July 1998 and this has involved a number of public meetings.

In response to the concerns raised in public submissions with regard to gene technology and GM foods, ANZFA is in the process of preparing a public discussion paper on the safety assessment process for GM foods. This will be widely available and may assist in addressing some of the concerns raised by the public. Other government and industry bodies are also addressing the broader concerns in relation to gene technology.

12. *Maori beliefs and values*

Some New Zealand submitters stated that Maori people find genetic engineering in conflict with their beliefs and values and that, out of respect to Maori, no genetically modified foods should be allowed into New Zealand until a wider discussion, both within Maori and non–Maori, is held.

Evaluation

This issue was also raised during consideration of the proposal for the establishment of Standard A18. At that time, it was stated that the likely implications for Maori regarding genetically modified organisms surround the issues of the rights of Maori to the genetic material from flora and fauna indigenous to New Zealand and the release into the environment of genetically modified organisms. The *HSNO Act 1996* requires that these matters be considered by ERMA.

13. *Environmental concerns and the broader regulatory framework*

A number of submitters have raised concerns that genetically modified crops may pose a risk to the environment.

Evaluation

These issues are considered in the assessment processes of GMAC in Australia and the Environmental Risk Management Authority (ERMA) in New Zealand. The Authority does not have the mandate to assess matters relating to environmental risks resulting from the release of food produced using gene technology into the environment. However, links exist between ANZFA and other regulatory agencies in both Australia and New Zealand, and a large degree of information sharing occurs. ANZFA would not recommend the approval of a food produced using gene technology if the genetically modified organism from which it was derived did not have the appropriate clearance for general release from either GMAC (or its successor) or ERMA, as appropriate.

The regulatory system in Australia will comprise the existing regulators with a legal remit to cover some aspects of GM products (such as imports, food, agricultural and veterinary chemicals):

- the Australia New Zealand Food Authority (ANZFA)
- the Therapeutic Goods Administration (TGA)
- the National Registration Authority for Agricultural and Veterinary Chemicals (NRA)
- the National Industrial Chemicals Notification and Assessment Scheme (NICNAS)
- the Australian Quarantine and Inspection Service (AQIS).

Similarly, various other departments and agencies play their role in the regulatory process in New Zealand:

- the Ministry of Agriculture and Fisheries (MAF)
- the Ministry of Health (MoH)
- the Ministry of Research, Science and Technology (MoRST)

In Australia a new Office of the Gene Technology Regulator (OGTR) will complement the existing arrangements. OGTR will supersede the existing arrangements under the Genetic Manipulation Advisory Committee (GMAC), which advises on research and environmental release of GMOs. OGTR will regulate all GMOs and any 'gap' products (i.e. products for which no other regulator has responsibility).

All GM food is assessed and regulated by the Australia New Zealand Food Authority (ANZFA) under the direction of Commonwealth, State and Territories Health Ministers and the New Zealand Health Minister, sitting as Australia New Zealand Food Standards Council (ANZFSC).

There will be an interface between ANZFA and OGTR. Consequential amendments proposed to the ANZFA Act arising from the draft Gene Technology Bill 2000 will establish a statutory interface between OGTR and ANZFA. This will involve amendments to the ANZFA Act requiring the Authority to advise OGTR of recommendations to ANZFSC regarding the standard for foods produced using gene technology (currently Standard A 18).

14. Maximum residue levels of agriculture/veterinary chemicals

A number of submitters have raised concerns that residues of agricultural and veterinary chemicals in genetically modified (e.g. herbicide tolerant) crops may pose a health risk.

Residues of these chemicals can only legally be present if the chemical has been registered for use in Australia and/or New Zealand, and it has been demonstrated that the residue at specified levels does not lead to adverse health impacts. The concentration of a chemical residue that may be present in a food is regulated through maximum residue limits (MRLs). The MRL is the highest residue concentration that is legally permitted in the food. Food products have to meet the MRL, whether or not they are derived from genetically modified organisms. The MRL does not indicate the chemical residue level that is always present in a food, but it does indicate the highest residue level that could result from the registered conditions of use.

It is important to note that MRLs are not direct public health and safety limits but rather, are primarily indicators of appropriate chemical usage. MRLs are always set at levels lower than,

and normally very much lower than, the health and safety limits. The MRL is determined following a comprehensive evaluation of scientific studies on chemistry, metabolism, analytical methods and residue levels. In Australia, the National Registration Authority (NRA) applies to ANZFA to amend the MRLs in the Food Standards Code and the application is considered by ANZFA through its legislated decision making processes. In New Zealand MRLs are set by the Ministry of Health， generally following a request from, and in collaboration with, the Ministry of Agriculture and Forestry. Only following demonstration that the use of agricultural and veterinary chemicals will not result in unsafe residues will the MRL enter into food law through its inclusion in either the Food Standards Code in Australia, or the Food Regulations (1984) in New Zealand.