**Application A549: Submission on the Draft Assessment Report from the Centre for Integrated Research in Biosafety**

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

The current submission from INBI asserts the following:

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

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

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

**FSANZ Response**

*General comments*

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

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

FSANZ's safety assessment of GM food is part of an overall risk analysis designed to identify whether a hazard, nutritional or other health and safety concern, is present in a GM food (hazard identification), and if present, to examine information on its nature and severity

(hazard characterisation). The hallmarks of this approach are: case-by-case assessment; consideration of both intended and unintended effects; and comparisons with conventional foods having an acceptable standard of safety.

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

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

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

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

*Comments on the INBI submission*

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

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

FSANZ has also noted that the INBI submission contains a number of factual errors. On more than one occasion, a journal article has been cited by INBI as evidence supporting a particular view, however when FSANZ has cross-checked the statements in the INBI submission with the cited article, the results and conclusions drawn by the author of the journal article are

contrary to those represented in the INBI submission. Such misinterpretations of the literature and speculative discussion have been used to give the erroneous impression of a heightened degree of uncertainty around the safety of food from LY038.

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

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

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

**References:**

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

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

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

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

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

*Response to the recommendations 1-94 from INBI*

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

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

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

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

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

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

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

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

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

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

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

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

**Reference:**

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

The Maillard reaction (also known as the browning reaction, glycation and non-enzymatic glycosylation) is a broad term that encompasses a wide range of reactions between sugars (carbonyl groups) and amino acids (free amino groups). These complex reactions produce hundreds of products, including those responsible for the cooked colour and flavour of many foods, such as bread crusts, chocolate, roasted meats and fried foods (McGee, 2004). Advanced Glycation End-products (AGEs) and Maillard reaction products (MRPs) are

produced during cooking, particularly frying and baking at high temperatures with low moisture. The particular range of MRPs produced will be influenced by the particular composition of proteins, sugars and fat of the food and also by the cooking method and duration of cooking. The Maillard reaction also occurs *in vivo* and during prolonged storage of food.

There is conflicting evidence for the health benefits or harm due to dietary MRPs, reflecting the wide range of Maillard products that exist. For example, Kitts and Hu (2005) suggest the antioxidant activity of MRPs can have a protective effect on cells, as well as enhancing food shelf life. The MRPs in bread crusts, notably pronyl-L-lysine and N-epsilon carboxymethyllysine, have also been shown to enhance antioxidant capacity and lead to an increase in chemopreventive enzymes (Somoza *et al*., 2005). In contrast, recent studies have found that the presence of acrylamide, a known carcinogen, in some fried foods is due to the reaction of the amino acid asparagine with sugars (e.g. Becalski *et al*., 2004). There is still no clear link between dietary acrylamide exposure and cancer incidence, despite the long history of consumption of browned foods (Blank, 2005). The possible health effects of acrylamide in food are areas of ongoing research.

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

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

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

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

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

Furthermore, the increased levels of lysine in LY038, and their potential to form AGEs, should be considered in the context of the total diet. Although the levels of lysine in LY038 are significantly increased (almost doubled) compared to conventional corn, corn is a poor source of lysine. Even if all corn products consumed by Australian and New Zealander consumers were derived from LY038 corn, this would represent an insignificant increase in lysine consumption as Australian and New Zealand populations consume only relatively small quantities of corn-derived products.

Data from the 1995 Australian National Nutrition Survey (NNS) indicates that maize was consumed in the form of maize flour by 2394 consumers (17% of the 13858 survey respondents). Consumption for Australian maize consumers aged 2 years and above was 20 grams per day at the mean and the 95$^{th}$ percentile consumption for consumers was 67 grams per day[1]. For New Zealand, maize was consumed by 1066 consumers (23% of the 4636 survey respondents). Consumption for New Zealand maize consumers aged 15 years and above was 14 grams per day at the mean and the 95$^{th}$ percentile consumption for consumers was 60 grams per day.

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

**References:**

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

**Reference:**

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

The literature ranges used by the OECD, which represent information from a variety of sources from different years, are very useful where no other relevant data exist. However, in some cases, published literature ranges do not exist (e.g. for total dietary fibre in the case of maize proximate analysis, only a single value was available to the OECD at the time the consensus document was written).

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

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

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

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

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

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

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

Proximate analysis has been performed on LY038 corn kernels. FSANZ considers that this is sufficient information as a variety of different food products are produced from the kernels,

including starch, grits and flour. The constituents of the kernels are expected to be representative of the constituents of food derived from them.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Extract from Table 9 in A549 Safety Assessment Report

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

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

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

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

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

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

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

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

The INBI submission states that elevated levels of cadaverine in corn might have physiological effects including potentially leading to S-adenosyl-L-methionine (SAM) deficiency and the suppression of spermidine and spermine synthases. However, as the compositional analysis has shown that levels of cadaverine in both LY038 and LY038(-) are below the level of quantitation, there is no scientific basis for speculating that levels of SAM and spermidine might be altered.

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

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

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

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

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

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

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

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

unintended change in the modified plant. All variant forms of cDHDPS should be characterized for glycosylation or other posttranslational modifications (5.3.17).

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

The recent publications by the Applicant (Ruebelt *et al*., 2006a; Ruebelt *et al*., 2006b; Ruebelt *et al*., 2006c) referred to in the INBI submission detail an analysis of the proteome of *Arabidopsis thaliana*, comparing naturally occurring *Arabidopsis* lines with a variety of transgenic lines. However, the authors concluded "on the basis of the changes detected for the proteins surveyed, the genetic modification of *Arabidopsis* using three different genes and three different promoters did not result in any phenotypic or seed proteome differences exceeding the natural variation other than the intended differences due to the introduction of the transgene". Further comments were that "Not much change was seen here that would inform a safety assessment." Other studies have found similar results.

**References:**

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

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

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

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

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

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

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

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

See response to R43.

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

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

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

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

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

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

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

See response to R48.

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

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

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

See response to R47.

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

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

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

The issue of the potential for cDHDPS to form aggregates was addressed at Draft Assessment. Concern was raised by INBI because amyloid fibrils are involved in a variety of medical conditions such as Alzheimer's and Parkinson's diseases. However, these fibril aggregates are produced from endogenous proteins that have sustained mutations or have been misfolded, rather than from the consumption of particular dietary proteins.

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

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

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

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

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

Whether cDHDPS is located in the amyloplast/plastid (corn grain does not contain chloroplasts) or elsewhere in the cell is not a food safety issue. Furthermore, many of the products likely to be produced from LY038 are highly processed products (e.g. corn oil or

fructose syrup) and would not contain intact cells or organelles. cDHDPS is unlikely to be toxic or allergenic. It is not heat stable and is digested quickly.

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

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

See response to R.54

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

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

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

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

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

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

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

Even using the high intake of corn by Mexican people, alluded to in the INBI submission, intakes of lysine from LY038 would not be particularly high. For example, daily consumption of 350g LY038 corn (4800ppm lysine) would supply approximately 1.68g lysine. This is

equivalent to eating approximately 60g cheese or a similar amount of red meat. Corn intake in Australia and New Zealand is much lower (over 23 times less) than in Mexico.

From Draft Assessment Report Attachment 4:

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

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

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

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

**Reference:**

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

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

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

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

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

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

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

See response to R14 and R18.

R67: The studies should be conducted using animal models that are most appropriate for identifying harms relevant to people. Long-term (lifetime) studies should be included because high lysine corn is also high free lysine, saccharopine, α-aminoadipic acid, cadaverine and pipecolic acid corn. The Authority should report on chronic effects, evidence of carcinogens and co-carcinogens (AGEs have been linked to cancer Heijst et al., 2005), and proteins that are capable of forming aggregates. No structural analysis alone will predict the effect of context on an enzyme or its potential to produce unanticipated products in a novel context. Therefore, structural analyses equating *E. coli*- and *in planta*-produced cDHDPS cannot substitute for the use of *in planta*-produced cDHDPS in all biochemical and feeding experiments (NZIGE Submission section 5.3.7.2).

See responses to R14-16, R18 and R54.

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

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

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

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

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

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

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

See response to R42.

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

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

The Applicant conducted an *in vitro* digestibility study on the novel protein present in LY038 corn, cDHDPS, using a standardised protocol that has been shown to distinguish known allergens from proteins known not to be allergenic (Thomas *et al*., 2004). This protocol is not

intended to be an exact replica of conditions *in vivo*, but rather is used to compare the test protein to known allergens under the same conditions.

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

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

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

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

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

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

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

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

Analyses conducted as part of this study included: bodyweight and bodyweight gain; feed intake; feed conversion efficiency; carcass yield; meat composition; fat pad measurements;

and breast and thigh meat quality. All of these parameters (vigorous growth and carcass characteristics) are key indicators of the nutritional adequacy of the food.

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

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

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

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

The corn used in the rat feeding study was tested and confirmed to be >99.25% LY038 grain, therefore FSANZ does not agree that LY038 was 'diluted' in this study. However, testing indicated that <21% of the grain also contained the MON810 trait, suggesting that a minor proportion of the grain was LY038 x MON810. MON810 is an approved variety of GM corn and its presence is not anticipated to affect the outcome of the study.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

See response to R82-87.

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

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

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

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

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

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

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

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

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

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

One of the strengths of the current approach used by FSANZ and other regulators is the flexibility afforded by the guidelines, consensus documents, and case-by-case management of issues, which can accommodate the idiosyncrasies of each GM food. FSANZ sees strength in

using a process that reflects an international consensus based on a combined knowledge and expertise in assessing food-related risks.