

# **The role of animal feeding studies in the safety assessment of genetically modified foods**

**Report of a workshop hosted by Food Standards Australia New Zealand  
Friday 15<sup>th</sup> June 2007, Canberra**

## **EXECUTIVE SUMMARY**

Food Standards Australia New Zealand (FSANZ) convened an expert panel to develop guidance and recommendations on the role animal feeding studies using whole foods can play in the safety assessment of genetically modified (GM) foods. FSANZ hosted the workshop as part of our ongoing review of procedures for safety assessment to ensure that recent scientific and regulatory developments are reflected in our process. This report summarises the workshop presentations and panel discussion.

The expert panel made a number of recommendations.

### **The panel acknowledged:**

1. the inherent compositional variability of all foods and noted that a scientifically-informed comparative assessment of GM foods against their conventional counterparts can generally identify potential adverse health effects or differences requiring further evaluation.
2. that whole-food animal feeding studies may be informative in some limited circumstances, but these studies need further refinement in relation to experimental design.
3. that properly designed animal feeding studies may be of most value in identifying health-related thresholds for intended effects on food composition.

### **The panel recommended that FSANZ:**

4. Continue to assess GM foods on the basis of best available science.
5. Consistent with existing FSANZ published guidelines, continue a case-by-case analysis of what data will be of most use for safety assessment of GM food.
6. Where the results of relevant animal feeding studies are available, evaluate them with critical attention to the methodology and potential limitations in interpretation of these types of studies.

The recommendations of the expert panel will be considered by FSANZ in assessing whether there are sound scientific arguments for refining the current approach to safety assessment of GM foods.

## **BACKGROUND**

All GM foods in Australia and New Zealand are required to undergo a safety assessment conducted by FSANZ before they may be approved for sale. The current approach to safety assessment focuses on the concept of comparison to a conventional counterpart with a history of safe use, and is consistent with international principles and guidelines developed by the Codex *Ad Hoc* Intergovernmental Task Force on Foods Derived from Biotechnology. The safety assessment aims to ascertain whether the GM food is at least as safe as the conventional counterpart food.

FSANZ does not normally require feeding studies in animals to be included in the safety assessment of GM foods. As part of FSANZ's regular review of its safety assessment procedures, FSANZ convened an expert panel to develop guidance and recommendations on the role animal feeding studies can play in the safety assessment of whole GM foods. The panel included scientists with expertise in plant biology, toxicology, medical and veterinary science, and risk assessment. The composition of the expert panel is provided at **Appendix 1**. The panel was chaired by Professor Brian Priestly.

The workshop included a series of presentations designed to provide background and context for later discussion. Dr TJ Higgins and Dr Richard Richards discussed and compared the development of new plant products by both GM and conventional breeding. Dr Lisa Kelly provided an overview of the approach used by FSANZ to assess the safety of GM foods. Dr Ib Knudsen presented the results of recent European research aimed at developing and validating sensitive and specific methods to assess the safety of GM foods, particularly using 90-day rodent feeding studies, and discussed the present strengths and weaknesses of the rodent feeding model and potential for improvement. Dr Andrew Bartholomaeus discussed the traditional toxicological assessment of drugs and other chemicals.

The workshop considered the potential hazards of GM foods, how well those hazards are identified and assessed using the current approach, and the role animal feeding studies may play in either the identification or characterisation of those hazards. The panel considered the key issues and developed advice, with particular reference to the use and interpretation of animal feeding studies, for FSANZ to consider in reviewing the guidelines for the safety assessment of GM foods.

## **PRESENTATION SUMMARIES**

### Development of new plant products (Dr TJ Higgins and Dr Richard Richards)

The development of new plant products depends on genetic variability that may be inherent in the population, created by cross breeding, generated by mutagenesis, created by interspecific hybridisation or introduced through recombinant-DNA techniques.

Dr Richards described the evolution and development of wheat to exemplify some of these approaches. The wheat genome is the result of interspecific crosses that combined

three genomes, followed by centuries of conventional breeding. In addition, many important bread wheat cultivars in Australia contain alien chromosome segments. The transfer of large chromosomal segments, containing hundreds of genes, even from distantly related species, is commonly referred to as “chromosome engineering”. For example, a new wheat variety nearing commercial release displays viral resistance which has been introduced from a weedy wild grass. Many other crops also derive from interspecific crosses, including sugarcane and numerous fruits. Genetic variability generated by chemical mutagenesis can result in single nucleotide changes throughout the genome, whereas radiation mutagenesis frequently causes large deletions and rearrangements of the genome. Thus, substantial genetic variability can be introduced by conventional plant breeding techniques.

Desirable characteristics can also be introduced by transferring one or a few specific genes using recombinant-DNA techniques. Dr Higgins described the two methods that are commonly used to transfer DNA to plants. Biolistic methods for introducing DNA into plant cells involve bombarding plant tissues with tiny metal particles coated with DNA. The second method utilises a bacterium, *Agrobacterium*, which is naturally capable of transferring its own genes to plants, to transfer the desired gene into a plant cell. Both methods result in insertion of genes essentially randomly into the host genome. Plants are regenerated from cells that carry the foreign gene, or “transgene”. The resulting plants are then subject to further development and testing in the laboratory and glasshouse before progressing into field trials.

The development of both conventional and GM crops takes years, and many generations of plants, during which breeding lines are evaluated. A few lines that express the desirable traits are pursued while the majority of potential lines are discarded. It is agronomic traits that drive these selections, and the only food testing performed for conventional crops is for technological function, for example, dough or pasta quality.

Both conventional and GM plant breeding can lead to unintended effects<sup>1</sup>. Examples in conventional breeding include traits such as reduced yield or undesirable product colour. In the few cases where conventionally bred cultivars were found to have increased levels of undesirable compounds, such as glycoalkaloids in potatoes and cucurbitacin in squash and zucchini, these toxic compounds were already known to be present in those species, rather than being entirely novel compounds.

Dr Higgins presented a case study of an unintended effect in a GM plant; that of a bean alpha-amylase inhibitor expressed in GM peas that has a different glycosylation pattern. In addition, some of the novel protein is truncated. The native alpha-amylase inhibitor in other beans also shows a variety of native glycosylation patterns and C-terminal truncations. Unintended effects on the introduced protein are more likely where the introduced protein undergoes post-translational modifications. Some studies have found

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<sup>1</sup> The potential for unintended effects through both GM and traditional breeding is discussed in Cellini F, Chesson A, Colquhoun I, Constable A, Davies HV, Engel KH, Gatehouse AM, Kärenlampi S, Kok EJ, Leguay JJ, Lehesranta S, Noteborn HP, Pedersen J, Smith M. 2004. Unintended effects and their detection in genetically modified crops. *Food Chem Toxicol.* **42**: 1089-1125.

that there are larger differences in gene expression between two non-GM lines than in comparisons of GM and non-GM lines<sup>2</sup>.

In discussion following the presentations, it was noted that new crops developed without recombinant-DNA techniques are not generally subject to regulatory oversight, although in Australia and New Zealand, there is scope for foods that have been significantly altered to be captured under novel food regulations.

#### The current approach to safety assessment of GM foods (Dr Lisa Kelly)

Dr Kelly began her presentation by introducing the concept that food is not inherently safe, but rather is presumed to be safe based on human experience. For foods with no history of safe use, such as GM foods, where there is no presumption of safety, a more formal risk assessment process is used, relying on a comparison of the new GM food to a conventional counterpart that has a history of safe use. This does not identify all hazards, but aims to identify any new or altered hazards relative to the conventional counterpart. These are then subject to further assessment to determine their safety. The goal is to determine whether the GM food is comparable to the conventional counterpart food in terms of its safety.

The safety assessment of GM food conducted by FSANZ includes a detailed characterisation of the genetic modification to the plant; a characterisation of any novel proteins, including their potential toxicity and allergenicity; and a consideration of the composition and nutritional adequacy of the food, including whether there had been any unintended changes to the food. Although acute toxicity studies on the isolated or purified novel protein are routinely undertaken, their relevance is questionable, as the results of other studies often indicate that the novel protein is rapidly degraded by stomach proteases, so there is unlikely to be any systemic exposure in the test animal.

The safety assessment approach applied to GM foods is modified from the traditional risk assessment of single chemicals (e.g. food additives) that is used to derive safe levels of exposure (intake). Such techniques are not necessarily applicable to whole foods, which are complex mixtures of chemicals that have often not been fully characterised. There are potential problems with nutritional imbalances from overfeeding of a single food; and it is difficult to achieve large multiples over anticipated human intake levels. This can limit the sensitivity of these studies, and interpretation of any adverse effect is complicated by the difficulty in attributing the effect to any specific food component.

In practice, the primary objective in the assessment of GM foods is to look for differences (intended and unintended) in comparison to the conventional counterpart. Identification of “differences” does not necessarily imply the food is less safe. Each difference must be evaluated for its potential impact on the safety of the food. The intent of the assessment is to reach a conclusion about the safety of the GM food under its intended conditions of use, based on the totality of the evidence.

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<sup>2</sup> Shewry PR, Baudo M, Lovegrove A, Powers S, Napier JA, Ward JL, Baker JM, Beale MH (2007) Are GM and conventionally bred cereals really different? *Trends in Food Science & Technology* **18**: 201-209.

The safety assessment considers both intended and unintended effects of the modification. Unintended effects can arise from the insertion of DNA into the genome; expression of the new trait; or subsequent conventional breeding steps. As no single test can detect all possible unintended effects, or identify those relevant to human health, a variety of data and information must be used. There are various examples of unintended effects that raise concerns to public health and safety that have been detected during the research and development stage and therefore did not proceed to market, for example, a gene from Brazil nut transferred to soybean that was found to be allergenic. Questions are often raised about the ability to detect all possible unintended effects using the current approach, and other studies that are often suggested to detect unintended effects include: animal toxicity studies; profiling techniques; and, post-market monitoring (long term effects).

#### The case for GM food animal feeding studies (Dr Ib Knudsen)

Dr Knudsen presented the findings of a European research project, known as the SAFOTEST project, conducted from 2000 to 2004, which had the objective of improving the sensitivity and specificity of GM food safety assessment. The project was particularly focussed on improving the standard OECD 90-day rodent study. As many previous tests of GM foods using the conventional 90-day study had not produced positive results, it has not been clear whether such tests are able to detect differences between GM and non-GM foods.

The SAFOTEST model combines a detailed characterisation of the GM plant, including information about the molecular characteristics (gene construct, site of insertion), chemical characteristics of the gene product (short term in vivo and in vitro studies), compositional analysis (both targeted and non-targeted through metabolite profiling) with a focus on unintended changes. This data informs the design of a 90-day feeding study. The 90-day rodent studies are intended to combine nutritional and toxicological information. The 90-day study in the SAFOTEST approach is not intended as a toxicity study, but a comparative safety study, which establishes the comparative safety between the GM food and its traditional counterpart. Therefore the 90-day feeding study in SAFOTEST only uses one control group and one dose group, both receiving the highest nutritionally tolerable intake level.

Dr Knudsen described the study of a GM rice expressing a kidney bean lectin agglutinin E-form (PHA-E lectin) to highlight key features of the SAFOTEST protocol<sup>3</sup>. The PHA-E protein is known to have high mammalian toxicity. Part of the aim of the study was to establish whether a 90-day rodent feeding study could have sufficient sensitivity to detect the toxicity of this protein expressed in the whole GM food. The parental rice is tested against GM rice to determine whether they have the same level of safety. Another test group with both GM lectin plus spiked lectin PHA-E is incorporated to identify any

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<sup>3</sup> Poulsen M, Schrøder M, Wilcks A, Kroghsbo S, Lindecrona RH, Miller A, Frenzel T, Danier J, Rychlik M, Shu Q, Emami K, Taylor M, Gatehouse A, Engel K-H, Knudsen I (2007) Safety testing of GM-rice expressing PHA-E lectin using a new animal test design. *Food Chem. Toxicol.* **45**: 364-377.

effects that are due to the novel protein itself, thus distinguishing any effects that are due to unintended effects. Any identified novel unintended effects in the animal test may be the subject for additional studies.

Purified PHA-E protein was tested at three dose levels in a 28 day preliminary toxicity study to determine a Lowest-Observed-Adverse-Effect-Level (LOAEL) and identify possible dose-dependent effects. This information was used in the design of the 90-day study. Statistically significant differences between the animals fed the test and control diets were seen for some parameters such as small intestine, pancreas and stomach weight and plasma biochemistry. The results from the 90-day study repeat the findings of PHA-E effects seen in the 28-day study. The spiking with pure PHA-E increases the biological effects of the inherently produced PHA-E in a dose dependent manner, so effects observed in both test groups confirm the *specificity* of the 90-day study. The effects identified in the PHA-E rice group confirm the overall *sensitivity* of the 90-day feeding study in identifying both the intended changes (PHA-E) and potential unintended changes in the GM food.

Dr Knudsen emphasised that an important feature of SAFOTEST is the detailed compositional analysis of the whole food prior to commencement of the feeding study. This allowed adjustment of the diet for the study animals, including the incorporation of rice at 60% of the basic purified diet while maintaining nutritional balance. A more recent NOFORISK study was able to incorporate GM potatoes into a hamster diet at 20%, 40% and 60% of the total diet. This is a significant advance on many earlier studies where test material was limited to around 5-10% of the diet to avoid nutritional imbalances. Also, there is now widespread recognition that the standard lab rat diet provides an excess of nutrients that may mask deleterious unintended nutritional and toxic consequences of the GM food and the diet including the added GM food used in SAFOTEST and NOFORISK is therefore being modified to be a *de minimis* diet optimising the possibilities for detecting unintended effects of the GM food. Further development of the design and use of purified rodent diets with an optimal nutritional composition may further improve their use in whole food feeding studies.

Separately to the SAFOTEST programme, a working group of the European Food Safety Authority (EFSA) is currently considering the role of animal feeding trials in the safety and nutritional assessment of GM plant derived foods/feed and are discussing *the overall sensitivity, specificity and predictivity of subchronic animal feeding tests* based upon surveys of animal testing in the scientific literature. The Working Group are currently finalising the report that will be delivered to the EFSA GMO panel, following open consultation on a draft report<sup>4</sup>. The draft report has proposed a tiered approach to the use of the 90-day rodent feeding studies depending on the nature of the genetic modification. Modifications where the novel protein is totally foreign and does not interfere with normal cell biochemistry, such as conferring herbicide tolerance or introducing an insect

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<sup>4</sup> EFSA (2006) Safety and Nutritional Assessment of GM Plant derived Foods/Feed: The role of animal feeding trials. Draft report for public consultation.  
[http://www.efsa.europa.eu/etc/medialib/efsa/science/gmo/gmo\\_consultations/gmo\\_animalfeedingtrials.Par.0002.File.dat/gmo\\_AnimalFeedingTrials\\_consultation.pdf](http://www.efsa.europa.eu/etc/medialib/efsa/science/gmo/gmo_consultations/gmo_animalfeedingtrials.Par.0002.File.dat/gmo_AnimalFeedingTrials_consultation.pdf)

toxin, may not warrant feeding studies. More complex modifications that deliberately alter cell function, such as conferring drought or salt resistance, may require a more thorough assessment that may include a feeding study. Deliberate modifications of the food composition, such as increasing the levels of nutrients, which may be more likely to alter other metabolic pathways would require the most thorough analysis.

Dr Knudsen described the results of a study of the capacity of the standard 90-day feeding study to detect unintended effects (Cockburn, EFSA, 2006). The study drew on an earlier report<sup>5</sup> that compiled results from 121 chemicals administered orally and determined Lowest-Observed-Effect-Levels (LOELs) ranging from 0.2 mg/kg bw/day to 5000 mg/kg bw/day with a mean LOEL of 100 mg/kg bw/day. Using this mean LOEL and knowing the amount of whole food in the diet which contains putative toxicants, it is then possible to calculate the concentrations of toxicants necessary in the diet in order to be detected in these studies. By retro-fitting this data to various plant substances it is then possible to model the sensitivity of the rat subchronic feeding study for the detection of hypothetically increased substances such as anti-nutrients, toxicants or secondary metabolites. Assuming that a rat consumes on average 25 grams corn/kg bw/day in a 90-day study at a 33% dietary incorporation of corn in the diet, in order to expose the rat to 100 mg/kg bw/day (the calculated LOEL), the putative toxicant must be present in the 25 g corn at 4 mg/g corn (25g x 4 mg/g = 100 mg). Thus the concentration of this potentially toxic substance in corn equivalent to this LOEL is 4 mg/g corn, or 4000 mg/kg, or 0.4% in the corn grain. Thus, unintended changes may be detected in a 90-day rodent feeding study, however, it is unlikely that substances present in low amounts and with a low toxic potential will result in any observable effects.

A review of the capacity of the conventional 90-day rodent feeding study to predict toxicological effects in long term feeding studies compared findings from 3 and 24 month studies and found that the majority of toxicological findings in the 24 month studies were seen in or predicted by the 3-month subchronic tests. For those 2 year studies that detected unpredicted findings, the majority identified changes in organs that commonly show acute toxic effects, such as the liver, kidney and thyroid, suggesting that focus on these organs may increase the sensitivity of the 90-day study. Some comparative studies have also noted that the lowest and most conservative No-Observed-Effect-Level (NOEL) reported was derived from a 90-day subchronic study. In one study, the majority of NOELs derived from embryotoxicity and teratogenicity were higher than NOELs derived from subchronic studies.

Dr Knudsen concluded that the SAFOTEST and NOFORISK results indicate that a combination of molecular, chemical and biological data strengthens the overall safety assessment, and that animal feeding studies on whole foods designed on a case-by-case basis using a *de minimis* diet with well-defined ingredients and incorporating a spiking procedure has potential in adding value to the assessment of toxicity, nutritional efficacy and/or health promotion in single animal study. Dr Knudsen advocated that the

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<sup>5</sup> Munro IC, Ford RA, Kennepohl E, Sprenger JG. (1996) Correlation of structural class with no-observed-effect levels: a proposal for establishing a threshold of concern. Food Chem Toxicol. **34**: 829-67.

performance of a well designed 90-day feeding study with the whole food will improve the scientific background for a management decision significantly.

The potential value of these studies would be enhanced by having harmonised guidelines for the 90-day rodent feeding study with whole foods. Further research would help in establishing an optimal design (including endpoints to be assessed, number of test groups, inclusion levels, use of spiking) as well as the design and use of purified rodent diets to allow incorporation of large amounts of the test food while maintaining an optimal nutritional composition. The assessment tools for plant derived GM foods are likely to be applicable to the safety assessment of a range of functional and novel foods.

#### Toxicology in safety assessment of drugs and chemicals (Dr Andrew Bartholomaeus)

Dr Bartholomaeus noted that the primary purpose of dosing laboratory animals with chemicals is to characterise the hazard or intrinsic toxicity of a drug (or chemical) and thereby assist in predicting possible adverse events in humans. In order to define a chemical's intrinsic toxicity, the design and conduct of relevant toxicity studies in laboratory animals are important considerations and must take account of dose metrics and dose extrapolation, potential nutritional perturbation, the extent and range of parameters measured, the animal species selected, group size, and duration of study. While animal studies can be powerful tools in predicting effects in humans, the identified toxicological endpoints may not always be directly applicable to humans. Similarly, results from one test species, such as in mice, may not be predictive for another, such as rats.

Dr Bartholomaeus pointed out that, when interpreting the results of toxicity tests, a number of factors need to be taken into account. Repeat dose feeding studies in laboratory rodents are generally designed for actively growing young or pregnant animals, which represents over nutrition for adult animals especially in long term studies. The *ad libitum* feeding of rodents is inherently physiologically abnormal and may result in diseases associated with hyper-nutrition. Paradoxically rodents given the highest dose in carcinogenicity studies are often healthier and live longer because they have a lower body weight. This may confound the interpretation of these studies as the effect of lower body weight on increased survival relative to control rats must be considered before concluding whether there was a treatment-related effect.

A range of factors can alter body weight gain other than dietary intake and several measured parameters such as organ weights will change simply because of the body weight change. As rodents age they become heavier and their nutritional requirements decline so the concentration of a test compound which is incorporated into the feed will need to be adjusted to keep the dose per unit body weight constant over time. It is generally difficult to test compounds of low toxicity because it may require a substantial amount of the diet to be replaced with the test compound in order to get a concentration which provides evidence of toxicity.

The experimental design should aim for the highest dose that has an effect but that is minimally toxic. Any observed dose response effect needs to be greater than the expected biological variability among groups. If no effect is seen in the test groups, the study is uninterpretable as no toxicological endpoint that may be of concern can be defined. If toxicity has been demonstrated then the duration of an animal study should extend beyond the predicted duration of human exposure. Testing a range of doses over a long time serves to magnify the potential toxicological response in the animal.

The interpretation of toxicological findings, even in well designed studies, is often complex. The analysis must consider the relevance of particular findings for the particular test species and for humans. Considerations must include identifying concordance, correlation and biological plausibility, which together lead to a convincing conclusion. Badly designed or reported toxicological studies are potentially entirely uninterpretable and should be disregarded for risk assessment purposes.

Statistical analyses of data are not always relevant and can often confound the interpretation rather than assist. Small statistical differences are usually grossly outweighed by the extrapolation between species (ie. rat to human uncertainty is far greater than anything that can be controlled in statistical analysis). In general, if statistical analyses are required to detect small differences, the change probably isn't toxicologically important. Conversely, a lack of statistical significance should not outweigh concordance and biological plausibility. For example, for highly variable physiological parameters, it may be impossible to discriminate between random differences and a treatment-related effect if there are insufficient test groups and an absence of a dose response. Conversely, for tight physiologically controlled parameters, even small differences may be treatment related and biologically significant, even if not statistically significant. Low frequency effects that are not statistically significant may, when combined with results from a number of species and a number of time points, provide correlation and concordance that builds a case for an effect which is relevant in humans. Clearly, the more animals in a group, the greater the power, and the less likely it is that random effects will distort the outcome. However, there is now a general trend towards using fewer laboratory animals per test group largely based on cost imperatives. This has the consequent effect of complicating interpretation of the results.

A common strategy to deal with biological variability in long-term drug toxicology studies is to incorporate two control groups to highlight the background natural variation. Statistically significant differences between control groups are almost always seen and always expected, partly because of the high number of endpoints being tested, and despite the large group sizes (60+ per sex per group).

Dr Bartholomaeus emphasised the need to distinguish between statistical, biological and toxicological significance. Biologically significant effects may not be toxicologically significant, for example, a change in liver weight due to a change in xenobiotic load, may not be an adverse effect in its own right. These may have toxicological consequences, for example, increased/decreased detoxification of a chemical, but may not in itself be a toxicological endpoint. Even a clear treatment related adverse effect may not be

toxicologically significant, for example, pregnant rabbits are sensitive to handling and will often stop eating. If this occurs during organogenesis, the developing embryo/fetus will be nutritionally deprived, so any adverse effect observed in the fetuses will be due to the handling procedure rather than the administered chemical.

Dr Bartholomaeus concluded by making the point that laboratory animal studies which are unlikely to yield interpretable information are unethical. For example, in drug development, it is considered unethical to undertake an animal study with insufficient numbers per group because the random effects can distort their interpretation. Similarly long-term studies should not be undertaken until an adequate dose range finding study has been conducted.

## **SUMMARY OF PANEL DISCUSSION**

The chair initiated panel discussion on the effective use of science in the safety assessment and regulation of GM food. The panel agreed that a case-by-case assessment of new GM foods should remain a central tenet of the safety assessment.

### The current approach (the comparative approach)

The panel acknowledged the importance of the detailed analyses of a GM food prior to any consideration of conducting animal studies so that any studies can focus on specific potential hazards. From a practical and ethical point of view, a detailed molecular and biochemical analysis of the GM food is essential prior to any animal feeding studies. In this way, the comparative analyses can be used to optimise the design of any subsequent feeding study. The working group further considered that these analyses may be able to identify differences and eliminate them from concern based on prior knowledge. The need for any additional analysis through an animal feeding study can then be assessed case-by-case. Animal safety studies on whole foods have significant potential limitations and in many cases will not provide useful information. They are currently of limited use as regulatory tools.

There is no consistency globally in the use or requirement of animal feeding studies in GM food safety assessments. However, as some countries routinely require the results of feeding studies, even where the other analyses indicate the GM food is equivalent to the conventional food, results of such studies are often available. The working group did not consider that FSANZ should mandate the use of feeding studies, but recommended that when they are available, FSANZ should continue to assess those studies, taking particular note of the scientific validity of the study.

### The potential value of animal feeding studies

The panel noted that the refinements to the conventional 90-day study developed by the SAFOTEST and NOFORISK projects, particularly advances in overcoming nutritional imbalance and the use of spiking to provide some realistic dose response assessment of the intended change, are important refinements of these types of tests for assessing whole

foods. While acknowledging the usefulness of the SAFOTEST protocol as a research tool, the panel did not reach a consensus regarding the usefulness of the studies as a regulatory tool.

The capacity of animal feeding studies to detect unintended and unidentified changes in whole foods was explored. The results of the SAFOTEST project demonstrate that the refined 90-day feeding study is sufficiently sensitive to detect the toxicity of the GM and spiked PHA-E lectin. However, it was noted that, in this case, as the PHA-E lectin is well known to be toxic, the classic toxicological kinetics, modelling and dose response studies were done to refine the whole food feeding study, effectively making this a classic toxicity study within a different dietary matrix. Where the substances at issue are already known, there is more to be gained by assessing those directly than in a whole food. Where the intended change is a novel protein that is known to be readily digested and without systemic exposure, spiking will not be informative.

The potential value of the refined 90-day study is any ability to assess the hazard of unknown components of a GM food. However, substances present in low amounts or with a low toxic potential are unlikely to result in any observable effects. Historical data on conventional 90-day studies indicate that they have sufficient sensitivity to determine a LOAEL of 0.2 mg/kg bw/day, with the potential for further experimental design to increase the sensitivity further. The sensitivity and specificity will also depend on animal group sizes and the amount of food that can be added to the diet. The studies may be able to demonstrate a hazard but be unable to identify the exact cause of the hazard. However, while acknowledging the potential of these studies, concerns were voiced about the likelihood of them yielding interpretable and useful information. It may be that once enough is known about the changes in the food to conduct a feeding study well enough to avoid confounders, that level of understanding may make the study unnecessary.

#### Assessment of nutritional modifications

There was some discussion as to whether the conventional feeding study methodologies are sufficiently refined to tease out issues surrounding nutritional vs toxicological effects. Combining toxicological and nutritional assessment into a single study introduces complications and leaves little scope for dose escalation.

There is likely to be more value in feeding studies of nutritionally enhanced food, to establish nutritional adequacy and efficacy. However, it may be that human studies will be more predictive and ethical. GM animal feed is already routinely tested in animals, not to address toxicological concerns, but to assure farmers that the feed is suitable as the primary source of nutrition for their stock.

#### Consideration of other approaches

The panel discussion primarily focussed on the utility of a modified 90-day feeding study. In discussion of whether 90-day feeding studies are adequate to predict possible long term effects, there were differing views. It was acknowledged that the results of a

90-day study are often predictive, but also that the corroborative evidence provided by a long term study often assists in the interpretation of a 90-day study that on its own may be equivocal. If it becomes a requirement or general practice to conduct 90-day studies for GM food safety assessments, this would likely lead to calls for longer duration studies and studies of other toxicological endpoints (e.g., reproduction).

The panel acknowledged that profiling technologies may ultimately be refined sufficiently to assist in identification of unintended differences as part of the comparative assessment of GM foods. However, any differences identified would require further assessment of their potential safety concerns.

The capacity of post market monitoring to contribute to assessing health effects of GM foods was discussed. While post market monitoring produces useful sentinel data on drug safety and adverse effects, in this case, people who provide a detailed history are taking a highly defined substance where there is already an idea of the types of adverse health effects that may be found. In contrast, any post market monitoring of GM foods would be of a population consuming different amounts at different times and in different ways amongst all other food intake, and with no particular health outcome in mind. If any health effects were vague or had a high background rate in the population any adverse effects could not be attributed to a particular cause. These factors make it unlikely that an adverse health effect due to a GM food could be detected above all the other health effects in the general population. There is also a valid public expectation that food safety should be adequately assessed before the food is placed on the market.

#### Final remarks

Given that in many cases there is no prima facie evidence of hazard and the GM food is already so well characterised, there will often not be a sufficient case to conduct animal feeding studies of GM foods. Several panel members expressed the view that the safety issues raised for GM foods are not likely to be different or greater than foods produced using conventional methods. Although conventional breeding and GM methodologies may achieve the same end, the intense scrutiny of GM foods is likely to continue. The issue of how to address this most effectively and economically is unlikely to be resolved by mandating studies that are difficult to conduct and open to misinterpretation.

## Appendix 1

### Expert Panel

- Chair, **Brian Priestly**, Head, Australian Centre for Human Health Risk Assessment and Professorial Fellow, Department of Epidemiology & Preventive Medicine, Monash University
- **Andrew Bartholomaeus**, Chief Toxicologist, Drug Safety Evaluation Branch, Therapeutic Goods Administration
- **Paul Brent**, (acting) Chief Scientist, FSANZ
- **Geoff Dandie**, Director, The Australian and New Zealand Council for the Care of Animals in Research and Teaching Ltd
- **Michael Dornbusch**, Manager, Evaluation Section, Office of the Gene Technology Regulator
- **TJ Higgins**, Deputy Chief, CSIRO Plant Industry
- **Lisa Kelly**, Principal Scientist, FSANZ
- **Ib Knudsen**<sup>6</sup>, Chief Adviser in Food Safety and Toxicology, Denmark
- **Peter Langridge**, CEO, Australian Centre for Plant Functional Genomics
- **Utz Mueller**, Principal Toxicologist, FSANZ
- **Richard Richards**, Chief Research Scientist, CSIRO Plant Industry
- **Malcolm Sim**, Director, Monash Centre for Occupational and Environmental Health, Monash University

A small number of observers from FSANZ, the New Zealand Food Safety Authority and the Office of the Gene Technology Regulator also attended the workshop.

### Brief biographies of workshop chair and panel members

#### Chair: Professor Brian Priestly

Brian Priestly is a Professorial Fellow in the Department of Epidemiology & Preventive Medicine at Monash University. He took up the position of Director of the Australian Centre for Human Health Risk Assessment (ACHHRA) in December 2003. ACHHRA is a consortium of four Australian Universities (Monash, UQ, Flinders University and Griffith University) with expertise in toxicology and environmental health sciences. ACHHRA's objective is to provide a national focus for human health risk assessment, primarily in the area of food and environment pollutants, and to contribute to workforce development in HHRA.

Prior to leading ACHHRA, Brian was Director of the Laboratories Branch in the Therapeutic Goods Administration (TGA) from 2001-2003. From 1992-2001, he was Scientific Director of the chemicals toxicology and chemicals risk management programs of the Commonwealth Health portfolio. He had overall responsibility for toxicological assessment of pesticides and other toxic chemicals and he provided Health Department

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<sup>6</sup> Dr Knudsen participated in panel discussions, but, on principle, did not consider it appropriate as a foreign guest to make recommendations on Australia and New Zealand's approach to food regulation.

input into various national and international chemicals management programs. Brian was a member of the National Drugs & Poisons Committee for several years, he has Chaired the Advisory Committee for Pesticides & Health, and he has been active on several NHMRC technical committees and working groups over the past twenty years.

### **Dr Andrew Bartholomaeus**

Dr Andrew Bartholomaeus is Chief Toxicologist, Drug Safety Evaluation Branch of the Australian Therapeutic Goods Administration. He is also Head of the Drug Toxicology Evaluation Section, a group of 16 academic staff performing assessments of the preclinical (toxicology, pharmacology, kinetic) data for all new prescription medicines entering the Australian market. He previously held the position of Principal Toxicologist and Manager of the New Chemicals Assessment group within the Office of Chemical Safety, responsible for peer review of toxicology assessments performed internally and externally, policy development, liaison with industry, other government agencies and the OECD (1998-2004). Dr Bartholomaeus has held various toxicology positions within the TGA dealing with agricultural, veterinary, industrial, cosmetic, herbal and medicinal chemicals.

Dr Bartholomaeus is also an Expert Adviser to the toxicology panel for the WHO/FAO Joint Meeting on Pesticide Residues in Food and is a member of the Nanotoxicology Advisory Committee of the National Health & Medical Research Council. Dr Bartholomaeus holds a Bachelor of Pharmacy from the University of Sydney and a PhD in Toxicology from the Royal Melbourne Institute of Technology, Australia.

### **Dr Paul Brent**

Dr Paul Brent is acting Chief Scientist of Food Standards Australia New Zealand. Prior to this, Paul was Manager of Product Safety Standards section, responsible for risk management of a range of product safety standards, including novel foods, irradiated foods, genetically modified foods, food additives and contaminants. Dr Brent has represented FSANZ on GM food issues at several levels, including the Australia New Zealand Food Safety Ministerial Council. Dr Brent has been the Australian delegation leader to the UN/WHO Codex Committee on Food Additives and Contaminants for several years.

Dr Brent obtained his Bachelor of Science at Newcastle University and doctorate in Clinical Pharmacology at the University of Newcastle Medical School prior to working as a Research scientist in basic and clinical pharmacology, neuroscience and biochemistry. Prior to his appointment with FSANZ, Dr Brent worked as a toxicologist at the Therapeutic Goods Administration and has experience in the evaluation of animal and human toxicological data submitted for registration of agricultural, veterinary and industrial chemicals and in support of clinical trials.

### **Dr Geoff Dandie**

Dr Geoff Dandie is the Chief Executive Officer of The Australian and New Zealand Council for the Care of Animals in Research and Teaching (ANZCCART). Originally from Melbourne, he qualified with a Bachelor of Science with first class honours and a Ph.D. in Medicine from Monash University.

Prior to taking this position at ANZCCART, Geoff was a lecturer and researcher with a long-standing interest in experimental pathology, cell biology and immunology, and has lead research groups at the University of Tasmania and more recently at the Child Health Research Institute, in South Australia. He also has had extensive experience in administration and management in a range of organisations and in conference organisation and publication. Importantly, Geoff came to ANZCCART with a great deal of experience working as a member and chair of animal ethics committees, as well as in the education and training of students and staff in the care of animals in research and teaching and related ethics issues. Geoff has also spent a couple of years working in the Biotechnology Industry, where he was involved with the development of high throughput bioassays that effectively allowed tens of thousands of samples to be tested for potential bioactivity per day - effectively replacing huge numbers of animals that would otherwise have been used for screening bioactive fractions.

During the past two years, Geoff has also been working with the Australian Federal Government on behalf of ANZCCART as a part of the team involved with implementing the Australian Animal Welfare Strategy, which aims to improve the welfare outcomes for all Australian animals.

### **Dr Michael Dornbusch**

Dr Michael Dornbusch is currently Manager, Plant Evaluation in the Office of the Gene Technology Regulator (OGTR). The Plant Evaluation section conducts human health and environmental risks assessments for dealings involving the intentional release of genetically modified plants into the Australian environment.

Michael received a PhD in biochemical toxicology from the University of New England in 1994 and undertook research and development work for a novel plant-based medicine. Michael has worked in risk assessment and regulatory science since 1997 when he joined the National Registration Authority for Agricultural and Veterinary Chemicals (now the Australian Pesticides and Veterinary Medicines authority). Since then he has worked on toxicology and human health risk assessments with the Office of Complementary Medicines and the Office of Chemical Safety, both of which are a part the Therapeutic Goods Administration. Michael joined the OGTR in 2003.

### **Dr TJ Higgins**

Dr Thomas J. Higgins is currently a Chief Research Scientist and Deputy Chief of CSIRO Plant Industry. His major research focus is the application of gene technology for plant improvement. He is particularly interested in improving the nutritive value of plants for feed and food uses and protecting plants from pests and disease. Dr Higgins is a strong advocate of science communication and regularly discusses gene technology in public forums.

Dr Higgins holds a Bachelor of Agricultural Science, National University of Ireland, 1967 and PhD, University of California, USA, 1971. Dr TJ Higgins first came to Australia as a postdoctoral fellow with the Research School of Biological Sciences at the Australian National University, in Canberra, Australian Capital Territory. He joined CSIRO Plant Industry shortly thereafter. In 1981, he returned to the United States for one year as a Howard Hughes Medical Institute Fellow at the University of Washington, Seattle.

### **Dr Lisa Kelly**

Dr Lisa Kelly is a Principal Scientist in the Risk Assessment – Chemical Safety Section of Food Standards Australia New Zealand (FSANZ). Lisa's primary area of expertise is in the safety assessment of GM foods, and she also has experience in assessing the safety of chemicals in food (food additives, contaminants, nutrients).

Lisa has considerable international experience in the GM food area - she is currently the Chair of the OECD Task Force for the Safety of Novel Foods and Feeds and also leads the Australian delegation to the Codex Ad Hoc Intergovernmental Task Force on Foods Derived from Biotechnology, where Australia has been leading the development of a new Codex guideline for the safety assessment of food derived from GM animals. Lisa is also a member of the FAO/WHO Expert Panel on Biotechnology and Food Safety and recently attended, and acted as rapporteur for, the FAO/WHO Expert Consultation of the Safety of Foods Derived from Recombinant-DNA Animals.

Lisa received a PhD in molecular plant virology from the Australian National University in 1994 and undertook post-graduate research in plant biotechnology and recombinant antibody technology at CSIRO Plant Industry up until 1997, when she joined FSANZ.

### **Dr Ib Knudsen**

Dr Ib Knudsen is a consultant Chief Adviser in Food Safety and Toxicology, having recently retired as Chief Adviser in Food Safety and Toxicology in the Department of Toxicology and Risk Assessment, Danish Institute for Food and Veterinary Research, Ministry of Family and Consumer Affairs, Denmark (2002-2006). In this role, he was responsible for general national and international risk assessment and research initiatives within the Department. Dr Knudsen coordinated and participated in EC-funded R&D projects in the fields of genetically modified foods (SAFOTEST, see WG1 in

[www.entransfood.com](http://www.entransfood.com) ) as well as novel and functional foods addressing food safety, risk-benefit assessments using new approaches in *in-vitro* and *in-vivo* testing and applying probabilistic, genomic and profiling techniques (NOFORISK, see [www.NOFORISK.org](http://www.NOFORISK.org) ), and participation in SAFE FOODS dealing with general risk assessment ([www.safefoods.nl](http://www.safefoods.nl) ).

Dr Knudsen previously held positions of Executive Director of the Institute of Food Safety and Toxicology, Danish Veterinary and Food Administration, Ministry of Food, Agriculture and Fisheries (1987-2001) and Deputy Director of the Institute of Toxicology, National Food Agency of Denmark, Ministry of the Environment (1980-1987). Dr Knudsen has held personal membership of several national and international scientific advisory committees within food safety and food safety research, including membership of the Scientific Steering Committee of the European Commission from 1997-2003. He was Chairman for the Scientific Committee on Food of the European Commission from 1997 2000 and a Member of that Committee from 1988-2000. He was also a Member of the WHO Expert Advisory Panel on Food Safety from 1998-2006. Dr Knudsen holds a Doctorate in Veterinary Medicine from the Royal Veterinary and Agricultural University, Copenhagen (1966).

### **Professor Peter Langridge**

Peter Langridge is currently the Chief Executive Officer and Director of the Australian Centre for Plant Functional Genomics which was established in 2002 through funding from the Australian Research Council, the GRDC and the South Australian Government. The Centre focuses on tolerance to environmental stresses in wheat and barley. His research has focused on development and application of molecular biology to crop improvement. He served for six years on the Genetic Manipulation Advisory Committee and is currently a member of the Gene Technology Technical Advisory Committee of the Office of the Gene Technology Regulator. He is on the editorial boards of several international journals, member of the Steering Committee of the International Triticeae Mapping Initiative (ITMI) and serves on the Advisory Boards for the European Union BioExpoit Program, USA NSF Wheat Genomics Program, The Generation Challenge Program of the CGIAR and the Centre for Integrative Legume Research.

### **Dr Utz Mueller**

Dr Utz Mueller is the Principal Toxicologist and Manager of the Risk Assessment – Chemical Safety Section in Food Standards Australia New Zealand (FSANZ). Prior to joining FSANZ in 2006 he was the Chief Scientist in the Office of Chemical Safety 2005-2006 with responsibility for toxicological assessments of pesticides.

Dr Mueller holds a Bachelor of Science (Hons) and PhD in Pharmacology from the University of Western Australia, Perth, WA. Dr Mueller was a Senior Research Fellow at Flinders University in South Australia prior to joining the Therapeutic Goods Administration (TGA) in 1996 where his primary task was the safety evaluation of pre-market therapeutic drugs. He subsequently joined the Office of Chemical Safety in 1997

to undertake pre-market safety assessments and review the safety of existing agricultural and veterinary chemicals. He has also been an advisor to the FAO/WHO Joint Meeting on Pesticide Residues (JMPR) for several years.

### **Dr Richard Richards**

Dr Richard Richards is the Program Leader of the High Performance Crops for Australia group. He is working to understand the genetic and physiological basis of the variation in growth, development and yield of wheat. He wants to use this understanding to breed higher yielding wheats. Elite wheat lines developed by Dr Richards and his group are currently in advanced yield trials throughout Australia. Several new wheat cultivars with higher yield potential are currently in commercial production in Australia.

Dr Richards completed undergraduate studies in science at the University of Melbourne, Melbourne, Victoria, Australia, prior to doing a Doctorate at the University of Western Australia, Perth, WA. Dr Richards was appointed lecturer in the Botany Department at the University of Western Australia, Perth, in 1975 and joined CSIRO Plant Industry in Canberra, Australian Capital Territory, in 1976. Dr Richards left CSIRO to join the University of California, Davis, California, USA, as a research agronomist, but returned to CSIRO Plant Industry in Canberra, ACT, in 1981.

### **Professor Malcolm Sim**

Malcolm is an occupational physician, who is Director of the Monash Centre for Occupational and Environmental Health (MonCOEH) in the Department of Epidemiology & Preventive Medicine, Faculty of Medicine, Nursing and Health Sciences at Monash University. His research program mainly comprises epidemiological studies of the role of workplace and environmental chemical and other hazards in chronic diseases in humans, such as cancer and respiratory disease, and he is the Chief Investigator for several cohort studies. He also has research interests in occupational disease surveillance, exposure assessment in epidemiological studies and veteran health research. Malcolm is an Investigator in the NHMRC-funded Australian Centre for Radiofrequency Bioeffects Research, the Australian Co-operative Research Centre for Water Quality and Treatment and the Australian Centre for Human Health Risk Assessment. He was an invited member of the Scientific Task Group on Inorganic Arsenic for the WHO's International Program on Chemical Safety.

Malcolm is an Associate Editor of the international journal, Occupational and Environmental Medicine and an elected member of the Management Group of the Scientific Committee for Occupational Epidemiology of the International Commission of Occupational Health. He sits on Research Advisory Committees for the National Centre for Environmental Toxicology, the Occupational Dermatology Research and Education Centre and the Australian Centre for Posttraumatic Mental Health. He was a member of a NHMRC Project Grant Review Panel in 2006, is a member of the Advisory Committee on Chemical Safety for the Office of Chemical Safety and a member of the Human Research Ethics Committee of the Cancer Council Victoria.

Malcolm has a medical degree from the University of Melbourne, an MSc in Occupational Medicine from the University of London and was awarded a National Health and Medical Research Council Fellowship to complete a PhD at Monash University.