Biotechnology risk assessment in Australia: A molecular perspective

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Biotechnology, especially the development of genetically modified crops, offers many potential economic, nutritional and environmental benefits, but equally many potential hazards and risks. Approval procedures for the release of genetically modified organisms are established under the Gene Technology Act 2000 (Cth). This article examines the methodology used to identify potential molecular risks associated with the use of biotechnology in food crop production. Evidence is presented which demonstrates the potential for molecular hazards to occur and brings into question the ability of risk assessment methods to identify these hazards. Despite appropriate regulations being in place, it appears that current hazard analysis procedures are not of sufficient sensitivity to ensure adequate protection for the Australian people and environment.

INTRODUCTION

The use of biotechnology[1] in agriculture offers the promise of higher yielding, more nutritious crops, with reduced reliance on pesticides and petrochemical fertilisers compared to traditional agricultural practices. In light of the significant environmental and health impacts that have resulted from current agricultural practices, crops with these qualities are certainly desirable. However, while there are enormous potential benefits for society, there is an ongoing debate surrounding the use of biotechnology for food crops. Consumers are anxious about both food and environmental safety, and a range of social and ethical issues.[2] Despite attempts by various intergovernmental agencies and governments to establish "rigorous and science based" assessment and approval systems, public acceptance does not seem to be forthcoming. Public pressure has forced major food retailers in the United States, United Kingdom and Asia to ban genetically modified (GM)[3] foods.[4] In Australia, a recent commitment by chicken producers to remove GM grain from chicken feed indicates the influence of public pressure here also.[5] Without full consumer acceptance, the potential of biotechnology as an agricultural tool will not be fully realised.

The *Gene Technology Act 2000* (Cth) (the Act) and the *Gene Technology Regulations 2001* (Cth) (the Regulations) established a mandatory, national enforceable licensing system for the use of gene technology in Australia. Although the Act was heralded as the foundation of a comprehensive

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[1] "Biotechnology" is an umbrella term used to describe a number of experimental protocols that are used to transfer highly modified genetic information (DNA) from one organism to another organism, eg from a bacteria to a plant or from a plant to a plant. The terms biotechnology and gene technology are used interchangeably in this paper.

[2] Australia, Senate Community Affairs Reference Committee, *A Cautionary Tale: Fish Don't Lay Tomatoes* (Australia, 2000) available online at http://www.aph.gov.au/senate/committee/clac_ctte/completed_inquiries/1999-02/gene/report/report.pdf (viewed 15 May 2006).

[3] Genetically modified (GM) crops are those that have been modified using biotechnology procedures.

[4] Hain M, Cocklin C and Gibbs D, "Regulating Biosciences: The Gene Technology Act 2000" (2002) 19 EPLJ 163 at 176.

[5] Bolt C, (2005) "Fears Force GM Chicken Feed from Chicken Menu", *The West Australian* (11 February 2005), p 5.

regulatory system,[6] it has resulted in very little real change with regard to the procedures which were already being used to evaluate risks that may be associated with the release of genetically modified organisms (GMOs). Reviews of the Act have highlighted several concerns. A discussion concentrating of the research and development aspects of the Act, noted that Australian biotechnology policy is "predicated on the assumption that the advantages in terms of economic competitiveness outweigh" the ethical, consumer and environmental concerns that may be associated with the use of biotechnology.[7] This view was reinforced in a paper that concluded the "comprehensive, independent and accountable" regulatory process promised by the Australian government has not been forthcoming.[8] The issue of environmental risk assessment under the Act has been addressed in a review of the risk assessment methodology applied before the release of GM crops into the environment.[9] It was concluded that "the theory and practice of risk assessment conducted by the Regulator is flawed".[10] This argument was supported by an evaluation of the Risk Assessment and Risk Management Plan (RARMP) for GM insect resistant cotton (Bollgard II) and GM insect resistant and herbicide resistant cotton (Bollgard II/Roundup ready).[11] The review highlighted several important issues, including that the scope of the environmental risk assessment was extremely narrow and limited to "the potential for weediness" and "the potential for gene flow to other organisms" and does not address the broader long-term ecological effects of introducing these crops.[12]

While previous authors have provided comprehensive reviews of the Act, its background, scope and limitations, they have not addressed the molecular issues. This paper provides a discussion of the molecular issues associated with using biotechnology to introduce genes into plant species. We relate this to the approach of the regulatory authorities with regard to identifying hazards associated with the use of biotechnology in food crops.

BIOTECHNOLOGY REGULATION: UNSUBSTANTIATED ASSUMPTIONS

The development of GM crops initially occurred more rapidly than the development of policies to authorise their commercial release. In 1990, no statutes were in place to specifically regulate the application of gene technologies. In response to community concerns, biotechnology companies felt that an assessment process would help build consumer confidence in GM food crops.[13] The challenge confronting regulatory authorities was whether a need existed for these crops to undergo risk assessments. Given that food crop species had long been grown and eaten without any evidence of harm, if no known toxins are produced by the inserted gene/s (transgene), was there any need to subject these crops to tests other than those normally carried out on new food plant varieties?

The issue was the subject of a joint meeting of the United Nations Food and Agriculture Organisation (FAO) and the World Health Organisation (WHO). The document resulting from this meeting, whilst recommending a new paradigm for safety evaluation, states that GM foods "will not pose any new risks as compared with those that might be expected from existing food sources …

[6] Australia, House of Representatives, *Debates* (22 June 2000) p 18104 (per the Hon Dr Michael Wooldridge, Minister for Health and Aged Care).

[7] Hain et al, n 4 at p163.

[8] Tranter M, "A Question of Confidence: An Appraisal of the Operation of the Gene Technology Act 2000" (2003) 20 EPLJ 245 at 259.

[9] Lawson C, "Risk Assessment in the Regulation of Gene Technology Under the Gene Technology Act 2000 (Cth) and the Gene Technology Regulations 2001 (Cth)" (2002) 19 EPLJ 195.

[10] Lawson, n 9 at 195.

[11] Office of the Gene Technology Regulator, *Risk Assessment and Risk Management Plan: Agronomic Assessment and Seed Increase in Eastern Australia of Transgenic Cotton Expressing cry1Ac and cry2Ab genes from Bacillus thuringiensis – DIR 005/2001* (OGTR, 2001).

[12] Lawson, n 9 at 208.

[13] Millstone E, Brunner E and Mayer S, "Beyond Substantial Equivalence" (1999) 401 *Nature* 525.

[produced] via traditional plant breeding".[14] The underlying assumption was that there is no difference between GM crops and their non-GM counterparts. At this time, there was no evidence upon which to base any conclusions.

Substantial equivalence

The need to benchmark novel products against one of known impact is a requirement of all impact assessment procedures. It allows meaningful comparisons to be made between one state and another; in the case of GM crops this means before and after the insertion of a new gene. Since humans have long grown and eaten traditional food crops, and GM plants are derived from these, the appropriate benchmark is the original crop plant.[15] The assumption is that if a new GM crop is demonstrated to be equivalent to the non-GM comparator there should be no increased risk associated with their use. The issue with this approach to risk assessment is not the use of non- GM crops as a comparator species; it is with the parameters chosen to establish that the GM crop is equivalent to the comparator species.

Under the model of substantial equivalence, which is the basis of GM crop assessment guidelines worldwide,[16] risk assessment starts with a comparative analysis of the GM plant with its non-GM counterpart. The purpose of the comparison is to identify any differences between them. A quantitative analysis of known toxins, allergens and selected nutrients, anti-nutrients, proteins, fats and carbohydrates is performed along with a comparison of features such as agronomic performance. This comparison leads to the plant being classified as:[17]

1. **Substantially equivalent**: there is no difference between the GM and its non-GM comparator;
2. **Substantially equivalent except for the introduced gene**: this is the usual finding with GM plants, in which case the protein/s produced by the newly inserted gene are tested for toxicological or allergenicity potential; or
3. **Not equivalent**: substantial differences identified, in which case the whole food/plant is further tested for toxicological or allergenicity concerns.

Once classified, any identified differences are then "subjected to a classical evaluation of their potential toxic, allergenic, or nutritional impacts".[18] However, if differences are not identified during the initial screening, they will not be detected in later stages of the assessment process, which concentrate on the products of the inserted gene and on confirming that known plant toxins are not produced in hazardous concentrations.

The detection of unknown toxins or allergens is therefore dependent upon these substances altering the composition of the few generic plant components that are tested. For example, plant production of a hazardous protein would need to result in significant alteration of the total protein content for a "red flag to be raised". Given that a plant species can produce up to 5000 different constituents,[19] including many toxins,[20] it is imperative that hazard identification is conducted using techniques that are accurate, sensitive and specific. Comparing components such as total proteins does not represent an assay of sufficient sensitivity to provide conclusive evidence that there is no difference in individual protein composition.[21]

[14] World Health Organisation (WHO), *Strategies for Assessing the Safety of Foods Produced by Biotechnology* (Report of Joint FAO/WHO Consultation, Geneva, 1991).

[15] Kuiper HA, Kleter GA, Hub P, Noteborn JM and Kok EJ, "Assessment of the Food Safety Issues Related to Genetically Modified Foods" (2001) 27(6) *The Plant Journal* 503 at 504.

[16] International Food Biotechnology Committee (IFBC), "Nutritional and Safety Assessments of Foods and Feeds Nutritionally Improved through Biotechnology" (2004) 3 *Comprehensive Reviews in Food Science and Food Safety* 1 at 45.

[17] Kuiper et al, n 15 at 504

[18] IFBC, n 16 at 45.

[19] Schubert D, "Sensible Regulations for GM Food Crops" (2005) 23 *Nature Biotechnology* 785.

[20] Ames BN, Profet M, Gold LS, "Dietary Pesticides (99.99% all natural)" (1990) 87 Proc Natl Acad Sci USA 7777.

[21] Corpillo D, Gardini G, Vaira AM, Basso M, Aime S, Accotto GP and Fasano M, "Proteomics as a Tool to Improve Investigation of Substantial Equivalence in Genetically Modified Organisms: The Case of a Virus-Resistant Tomato" (2004) 4 *Proteomics* 193; Millstone et al, n 13 at 525.

Australian GM Crop risk assessment: The Gene Technology Act 2000

The *Gene Technology Act 2000* appoints an independent statutory officer, the gene technology regulator (the Regulator), to oversee risk assessment, risk management and to make independent decisions on whether a GM crop is approved for commercial release in Australia. The Regulator (although supported by the Office of Gene Technology Regulator (OGTR), a Commonwealth regulatory agency) holds primary responsibility for ensuring that the Act is administered in such a way that the purpose is achieved.

The object of the Act is

> [T]o protect the health and safety of people, and to protect the environment, by identifying risks posed by or as a result of gene technology, and by managing those risks through regulating certain dealings with GMOs.[22]

The Regulator is therefore responsible for "identifying risks" in order to protect the health and safety of people and the environment. In this respect hazard identification is of vital importance to ensuring the object of the Act is achieved. Although the Australian regulators do not use the term "substantial equivalence", a compositional analysis is used to identify potential toxicity hazards. For example in the RARMP prepared for Bayer CropSciences InVigor([23] canola, the Regulator states that in its evaluation of potential hazards that may result in toxicity to humans and other organisms, a compositional analysis can "provide evidence of whether any unintended effects have been introduced into the GM canola lines as a result of the genetic modifications".[24] Short-term feeding studies are documented which provide information on acute toxicity issues but no evidence of chronic toxicity issues with this crop. The compositional analysis provides evidence to determine whether or not there are significant changes to total plant proteins but provides no evidence concerning altered levels of individual proteins.

While the stated object of the Act is broad, encompassing "risks posed by, or as a result of gene technology", the scope is considerably narrower. For example, references to the health and safety of people do not include ensuring that GM food is safe, even though any risks are a "result of gene technology".[25] The Regulator's role is largely limited to dealings with GMOs, not their products. While food safety is often stated as a responsibility of Food Standards Australia and New Zealand (FSANZ)[26], under the Act the Regulator "has the power to do all things necessary"[27] to perform the functions set out in s 27 of the Act. As such, the Regulator is "to provide information and advice to other regulatory agencies about GMOs and GM products".[28] In this respect, the Regulator has the power to offer advice to FSANZ with regard to the appropriate methodology to assess GM foods.

The Regulator assesses the risks of releasing GM crops into the environment, but the consideration of safety is limited to the potential risks that may occur because of such releases. This includes environmental safety issues, such as risks posed to farm workers through exposure to GM plant pollen, and to the potential for GM plants to be toxic to other organisms in the environment. In applications that have been previously been approved for food consumption by FSANZ, the Regulator considers the outcomes of FSANZ risk assessments in preparation of a RARMP.[29] A crop plant that has been previously found to be safe for human consumption is considered unlikely to be toxic to the environment. Therefore if the FSANZ risk assessment does not identify molecular risks it is likely that the results will also affect the outcome of the OGTR risk assessment procedures.

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[22] *Gene Technology Act 2000* (Cth), s 3.

[23] Office of the Gene Technology Regulator (OGTR), *Risk Assessment and Risk Management Plan: DIR 021/2002, Commercial Release of Genetically Modified (InVigor® hybrid) Canola* (OGTR, 2003).

[24] OGTR, n 23, p 59.

[25] *Gene Technology Act 2000* (Cth), s 3.

[26] OGTR, n 23, p 20.

[27] *Gene Technology Act 2000* (Cth), s 28.

[28] *Gene Technology Act 2000* (Cth), s 27(e).

[29] *Gene Technology Regulations 2001* (Cth), s 10(1).

Both agencies use a standard compositional analysis to demonstrate that there are no toxicity issues associated with new GM crops or foods derived from new GM crops. An illustration of how even significant changes to plant composition are not detected using this compositional analysis is provided in the following case study.

Case study: Genetically modified corn

Monsanto's Mon810[30] and Synergenta's Bt11[31] GM insect resistant corn are approved for human consumption in Australia and are grown commercially in North America, Canada and Argentina. These crops underwent the "rigorous science-based" health and/or environmental safety assessment processes of the countries in which they are grown or consumed. In Australia, FSANZ (formerly Australian and New Zealand Food Authority (ANZFA)) performed both risk assessments, using a range of criteria in the safety evaluations, including:

> characterisation of the transferred genes, analysis of changes at the DNA, protein and whole food levels, stability of the introduced genes, evaluation of intended and unintended changes and assessment of the potential allergenicity or toxicity of any newly expressed proteins.[32]

The levels of 18 amino acids were also tested and importantly, eight of these (44%) were found to be present in significantly higher concentrations in Mon810 corn compared to the control line. The comparison was broadened to include both the "literature range" for amino acids in corn and to other GM corn lines. Based on these comparisons it was concluded that these differences in amino acid levels were not "considered to represent a biological meaningful difference".[33] However, five years after passing the health and safety assessment procedures of the American Food and Drug Authority (FDA), and one year after they were approved for consumption in Australia by FSANZ, it was discovered that Mon810 and Bt11 corn stalks contain 33-97% more lignin than non GM corn.[34] The indigestibility of lignin has previously led to attempts with GE techniques to reduce its content in feed[35], yet inadvertently its content has been increased in one of the most important, and widely planted food crops.

Whilst significantly higher lignin content in corn stalks may, or may not, be a toxicity issue, there are concerns with these findings. Scientific understanding of the lignin biosynthetic pathway is incomplete;[36] however, it is a known product of the shikimic acid pathway, an extremely important biochemical pathway in plants. An estimated 40% of the dry weight component of plants are products of this pathway.[37] As far as could be ascertained from the published literature or from either the OGTR or FSANZ websites there have been no studies investigating whether or not this unintended effect is also apparent in corn kernels. However, evidence of significant differences in amino acid composition,[38] particularly the aromatic amino acids, tyrosine, phenylalanine and tryptophan, which are produced by the shikimic acid pathway, suggest potentially altered metabolism in the kernel also. This is a concern because, as Schubert notes, "components of this same biochemical pathway also

[30] Australia New Zealand Food Authority (ANZFA), *Food derived from insect-protected corn line Mon810: A Safety Assessment* (Technical Report Series Number 5, ANZFA, 2001) available online at http://www.foodstandards.gov.au/_srcfiles/TR5.pdf (viewed 15 May 2006).

[31] Australia New Zealand Food Authority (ANZFA), *Food derived from insect-protected, herbicide-tolerant Bt-11 corn: A safety Assessment* (Technical Report series No 10, ANZFA, 2001) available online at http://www.foodstandards.gov.au/_srcfiles/TR10.pdf (viewed 15 May 2006).

[32] ANZFA, n 30, p 3.

[33] ANZFA, n 30, p 15.

[34] Saxena D and Stotzky G, "Bt Corn Has a Higher Lignin Content Than Non-Bt Corn" (2001) 88 (9) American J Bot 1704.

[35] Sewalt VJH, Weiting NW, Blount JW, Jung HC, Masoud SA, Howle PA, Lamb C and Dixon RA, "Reduced Lignin Content and Altered Lignin Composition in Transgenic Tobacco Down-Regulated in Expression of L-Phenylalanine Ammonia-Lyase or Cinnamate 4-Hydroxylase" (1997) 115 (1) Plant Physiol 41.

[36] Kutchan TM, "Predictive Metabolic Engineering in Plants: Still Full of Surprises" (2005) 23 (8) *Trends in Biotechnology* 381.

[37] Freese W and Schubert D, "Safety Testing of Genetically Engineered Food" (2004) 21 *Biotechnology and Genetic Engineering Reviews* 299.

[38] ANZFA, n 30, p 15.

produce both flavonoids and isoflavonoids that have a high nutritional value, and rotenone, a plant-produced insecticide that may cause Parkinson's disease".[39] Furthermore, metabolic precursors to lignin biosynthesis are known to have detrimental effects in mammals. For example, caffeic acid, a lignin precursor, is known to cause forestomach tumors in male mice and in both male and female rats.[40]

Other direct or indirect effects resulting from the altered lignin content in these GM corn crops may become evident with further analysis. However, there is no evidence that issues such as these have been considered by either FSANZ or OGTR.

HAZARD IDENTIFICATION

The Act provides very little guidance on the process of risk assessment and risk management, and the Regulator has prepared detailed administrative guidelines outlining the approach to be used in the risk assessment process.[41] The *Risk Analysis Framework* (RAF) document outlines the principles of risk analysis developed to protect human health and safety, and the environment, in accordance with s 51 of the Act.[42] Risk analysis is defined in its broad sense as encompassing risk assessment, risk management and risk communication. Risk assessment is defined as the overall process of hazard identification and risk estimation.[43] The risk estimate is derived from both the "likelihood" and "consequence" of any identified hazard and is affected by uncertainty in either of these parameters.[44]A risk assessment matrix is generated, which provides estimates of the level of risk (negligible, low, moderate or high), which is then used to develop risk management strategies.

The importance of hazard identification in the risk assessment process is acknowledged in the phrase: "hazard identification underpins the process of risk assessment".[45] Likewise, the Regulator accepts that if hazards are not identified that they "may pose a major threat to health and the environment" and commits to the use of a "comprehensive approach" to hazard identification to ensure that the full range of hazards are identified.[46] The RAF does not prescribe a methodology to be used in the hazard analysis process, but refers specifically to investigating hazards posed by:[47]

- altered biochemistry;
- altered physiology;
- unintended change in gene expression;
- production of a substance toxic to humans;
- production of a substance allergenic to humans; or
- production of a substance that is toxic to, or causes ill health, or mortality, in non-target organisms.

The risks analysis approach used by the Regulator is described as employing the use of quantitative evidence and information, but it is also acknowledged that the approach will not rely on the use of a quantitative risk assessment.[48] The Regulator asserts that qualitative risk assessment is the most feasible approach to assess risks because:[49]

[39] Schubert, n 19 at 786.

[40] McGregor D, "Diets, Food Components and Human Cancer" (1998) 11 *Biotherapy* 189.

[41] Peel J, *The Precautionary Principle in Practice: Environmental Decision-Making and Scientific Uncertainty* (The Federation Press, 2005).

[42] Office of the Gene Technology Regulator (OGTR), *Risk Analysis Framework* (OGTR, 2005).

[43] OGTR, n 42, p 18.

[44] OGTR, n 42, p 18.

[45] OGTR, n 42, p 35.

[46] OGTR, n 42, p 35.

[47] OGTR, n 42, p 37.

[48] OGTR, n 42, p 28.

[49] OGTR, n 42, p 25.

- the types of organisms and types of introduced genes are highly varied and often novel;
- potential human health and environmental adverse effects are highly varied;
- environmental effects arise within highly complex systems that have many incompletely understood variables; and
- adverse effects may occur in the long term and are therefore difficult to quantify.

Although it is extremely difficult to quantify complex ecological interactions, rigorous analysis of plant composition will significantly improve the hazard identification process, thereby reducing the potential for negative ecological impacts to occur. As such, it is important that initial hazard identification procedures utilise methods that are unbiased, sensitive and specific. While compositional analysis is a quantitative procedure, the decision by the Regulator to use parameters such as whole proteins and fats to detect changes in plant constituents is highly speculative[50] as there is no evidence that this method of hazard identification provides any scientifically meaningful data. This severely undermines claims by the Regulator that information in the risk analysis process is assessed "against rigorous scientific standards."[51]

The question of whether the process of genetic engineering poses different potential hazards to traditional breeding practises underlies the molecular debate. Risk assessment procedures used in many countries, including Australia, hinge on the assumption that there are no increased risks associated with the use of biotechnology in plant breeding procedures.[52]

Efficacy of genetic engineering techniques

Industry proponents portray GE techniques[53] as a simple extension of traditional breeding practices. A common argument is that genetic engineering is a precise technology that allows a single well-characterised gene to be inserted into a new species.[54] The argument rests on the assumption that inserting one well-characterised gene results in less disruption and therefore less risk than in traditional breeding techniques. Traditional breeding techniques generally involve the recombination of thousands of genes between breeding pairs. By comparison, genetic engineering techniques involve inserting a gene (or genes), often highly modified using recombinant DNA technology, into the genome of a plant species. For example, if the desired outcome is herbicide tolerance in a plant, a gene producing an enzyme that degrades the herbicide is randomly inserted into the plant's genome.

The genetic engineering process used to introduce foreign DNA into a cell is known as transformation. In the creation of GM plants there are two main methods of transformation used, namely:[55]

- Microprojectile bombardment: which involves coating microscopic beads of tungsten or gold with the fragments of DNA constructed for insertion. The beads are then fired, using air pressure, into a culture of plant cells. The beads penetrate the cell wall and hopefully end up in the nucleus of the cell were they may be integrated into the cell's DNA. New plants are then regenerated from these cells; or
- T-DNA transfer: which involves the use of a soil bacterium that is responsible for causing crown gall in plants. This bacterium has the ability to penetrate the cell wall and insert sections of its DNA into its host's DNA. Scientists have utilised this ability to deliver DNA of interest into new cells. New plants are regenerated from these cells.

Two recently published studies have demonstrated that both transformation processes lead to mutations in the plant genome. One study investigated the effects of gene transfer by microprojectile

[50] Millstone et al, n 13 at 526; Corpillo et al, n 21 at 199.

[51] OGTR, n 42, p 45.

[52] IFBC, n 16 at 45.

[53] Miller HI and Conko G, "Precaution Without Principle" (2001) 19 *Nature Biotechnology* 302; IFBC, n 16 at 39.

[54] WHO, n 14 at s 4.2.1.

[55] Glick BR and Pasternak JJ, *Molecular Biotechnology: Principals and Applications of Recombinant DNA* (3rd ed, ASM Press, 2003) pp 517-523.

bombardment and demonstrated that regions of the plants DNA are rearranged and that extensive regions of plant DNA and smaller regions of the transgene had been scrambled during gene insertion.[56] In the second, research on the effects of the T-DNA transfer system found that the process results in both small and large-scale deletions and rearrangements of the plant's genes.[57] The research concentrated on analysing the whole genome and found both minor and gross alterations. Interestingly, many of these changes were localised to the area of the insert, but in several GM plants there was evidence of genome-wide structural change. While the resulting effects on plant metabolism were not studied, the authors conclude that these results need to be considered when characterising new GM crops.[58] Both of the GE techniques used to transfer genes into plants can result in extensive mutation, with unknown effects although the two studies have shown that many mutations are not necessarily lethal to the plant. Contrary to the stance taken by biotech industry proponents,[59] GM procedures are not precise and a cursory compositional analysis is not sufficient to demonstrate food and environmental safety.

It was long thought that genes in higher organisms such as plants and mammals were positioned randomly in the genome, however recent research demonstrates that DNA structure is more organised than previously believed. The first statistically vigorous analysis of complete genomes reported that genes are clustered in functional groups in the genome.[60] Many traits require the product of more than one gene, with genes required for the same trait (function) clustered together on the chromosome and often transferred between breeding partners in these functional clusters. Their position in the genome is not altered during breeding practices and the transferred genes are therefore in the same predictable locations on the chromosome. This is in stark contrast to genetic engineering procedures which:

- insert genes randomly into the genome, their final position could be anywhere in the genome of the cell;[61]
- can result in large areas of the plant's genome being deleted, duplicated or moved to a different place in the plant genome;[62]
- can result in two or more whole copies and also fragments of the transgene being inserted into the plant's genome;[63]
- can result in insertion of a transgene, or parts of transgene, into a plant gene, which has the potential to decrease or disrupt expression patterns of the plant's own genes;[64]
- have the potential to increase production of toxins not known to occur in the plant by activating silent genes or increasing production of toxins not yet identified in the plant species;[65]
- transfer genes into the cell that operate outside the cell's own regulatory mechanisms and are therefore always turned on, sometimes in every cell of the plant;[66] and

[56] Makarevitch I, Svitashev SK, and Somers DA, "Complete Sequence Analysis of Transgene Loci from Plants Transformed via Microprojectile Bombardment" (2003) 52 Plant Mol Biol 421.

[57] Forsbach A, Schubert D, Lechtenberg B, Gils M and Schmidt R, "A Comprehensive Characterization of Single-Copy T-DNA Insertions in the Arabidopsis Thaliana Genome" (2003) 52 Plant Mol Biol 161.

[58] Forsbach et al, n 57 at 162.

[59] Miller and Conko, n 53 at 302.

[60] Hurst LD, Pál C and Lercher MJ, "The Evolutionary Dynamics of Eukaryotic Gene Order" (2004) 5 *Nature Reviews Genetics* 299.

[61] Martineau B, *The Creation of the FlavrSavr® Tomato and the Birth of Biotech Food* (McGraw-Hill, 2001) p 69.

[62] Takano M, Egawa H, Ikedo J and Wakasa K, "The Structures of Integration Sites in Transgenic Rice" (1997) 11(3) *The Plant Journal* 353; Somers DA, Olhoft PM, Makarevitch IF and Svitashev SK "Mechanism(s) of Transgene Locus Formation" in Laing DH and Skinner DZ (eds) *Genetically modified crops: their development, uses and risks*, (Haworth Press, 2004).

[63] Gasson M and Burke D, "Scientific Perspectives on Regulating the Safety of Genetically Modified Foods" (2001) 2 *Nature Reviews Genetics* 217.

[64] Schubert D, "A Different Perspective on GM Food" (2002) 20 *Nature Biotechnology* 969.

[65] Schubert, n 64 at 969.

[66] Schubert, n 19 at 786.

- attach powerful promoter and enhancer sequences to the inserted gene to ensure the transferred gene operates in the new cell, but which have the potential to change the expression patterns of plant genes located near to them on the genome.[67]

These issues have lead to recognition that each new GM crop should be evaluated on a case by case basis as each gene insertion "event" has the potential to result in completely different outcomes.[68]

Unintended effects

Another argument of industry proponents is that inserting a gene of known function will reduce the chance of unintended effects occurring. There are however many examples in the literature illustrating that insertion of a gene with known function can result in completely unexpected results on plant metabolism.[69] Several possibilities may explain these results. First, as described previously, the insertion of genes by GE techniques can lead to unintended mutations of the plant genome, which can result in altered plant physiology. Given that single point mutations can lead to "complex responses at the level of the whole organism",[70] both transformation techniques carry a risk of mutation leading to unintended effects. Secondly, in the cells of all living organisms, genes are expressed in concert with many other genes, often with the product of one gene leading to the production of other gene products. When genes are transferred into a new plant, there is the potential for that gene product to interact with either plant genes or their products resulting in altered metabolism (pleiotrophic effects). This potential is impossible to directly predict and requires a more vigorous compositional analysis to identify than that currently conducted by the Australian regulators.

Industry proponents argue that product development and selection procedures involve processes that would eliminate the potential for these unknown/unintended effects to reach the marketplace.[71] It is true that many initial transformants (GM plants) are discarded during selection procedures, eg those plants that have either gross abnormalities, or other easily tested negative traits such as reduced agronomic performance. However, as illustrated with the GM corn case study, even large aberrations to plant metabolism can go undetected in selection procedures and subsequently in risk assessment procedures.

RISKS: LIKELIHOOD AND CONSEQUENCE

The likelihood of molecular hazard occurring

Very little definitive quantitative research has investigated the frequency with which unintended mutations occur during the gene insertion process. Likewise there is little research which examines the effects of gene insertion on the composition of a broad range of plant produced proteins or secondary metabolites. This is largely due to the fact that the technology with which to perform sensitive assays that detect a wide range of plant components has not been readily available. Recently however, there has been significant development in tools and techniques that provide more sensitive, comprehensive and unbiased analysis of gene expression patterns.[72] Published results generated using these techniques, suggest that altered plant metabolism is not uncommon.

A technique that uses gas chromatography and mass spectrometry (GC-MS) technologies to separate and analyze secondary metabolites was reported in 2001, in a study on previously

[67] Weigel D, Ahn JH, Bla'zquez MA, Borevitz JO, Christensen SK, Fankhauser C, Ferra'ndiz C, Kardailsky I, Malancharuvil EJ, Neff MM, Nguyen JT, Sato S, Wang Z, Xia Y, Dixon RA, Harrison MJ, Lamb CJ, Yanofsky MF, and Chory J, "Activation Tagging in Arabidopsis" (2000) 122 *Plant Physiology* 1003.

[68] Haselberger AG, "Codex Guidelines for GM Foods Include the Analysis of Unintended Effects" (2003) 21 *Nature Biotechnology* 739.

[69] Haselberger, n 68 at 739; Kuiper et al, n 15 at 516: Both authors provide examples of unintended effects.

[70] Fiehn O, Kopka J, Dörmann P, Altmann T, Trethewey RN and Willmitzer L, "Metabolite Profiling for Plant Functional Genomics" (2000) 18 *Nature Biotechnology* 1157.

[71] IFBC, n 16 at 48.

[72] Sweetlove LJ, Last RL and Fernie AR, "Predictive Metabolic Engineering: A Goal for Systems Biology" (2003) 132 *Plant Physiology* 420.

characterised GM potatoes.[73] The tested GM potatoes were genetically modified to have altered starch levels. Several findings were novel, and illustrate that gene insertion results in large changes to gene expression patterns in plant species. Results presented on 88 metabolites, 61 of which were identified in the study, demonstrated that the majority of the tested metabolites had significantly altered metabolism in the GM lines, when compared to the control potatoes. Most of these were due to changes in starch biochemical pathways, but there were also unexpected aberrations to amino acid levels and the shikimic acid pathway. Importantly, nine of the 88 metabolites were below the detectable level in the non-GM potatoes but detected in several of the GM lines. It was noted that; "[s]ome of these metabolites were observed in all of the transgenic types studied, whereas others were only present for a certain transgenic manipulation or even for a single transgenic line".[74] Of concern was one compound that could not be identified and was present in extremely high concentrations in one of the GM lines.

Another relevant paper notes that the use of similar techniques have demonstrated that that there were substantial differences between a GM and a non-GM potato line.[75] The authors concluded that the data has "important ramifications for the potential risk associated with transgenic organisms and theories of substantial equivalence". In contrast, similar methods were used to demonstrate that 95% of the low molecular weight compounds studies in a GM tomato were identical to those produced its non-GM variety.[76] These studies illustrate that GE techniques are not inherently dangerous, but that the potential for unintended effects to occur is real and can result in the production of metabolites not previously produced in the plant.

Protein expression studies using two-dimensional polyacrylamide gel electrophoresis (2-DE) allow proteins produced in plant cells to be separated by size and electrical charge. Rather than analyzing total proteins, which is the basis of current hazard identification procedures (as outlined previously), these techniques allow the simultaneous analysis of a large range of specific proteins, and their relative amounts, which can then allow a definitive comparison of protein expression patterns.[77] An advantage of this technique is that the identity of proteins is not required for the initial analysis. Any differences in protein levels are firstly identified using this relatively simple procedure, and any proteins of interest then extracted and identified. A problem with this approach is that it only provides a relative comparison and further tests would be required to quantify any observed differences.[78] The use of this technique to compare a GM and a non-GM tomato plant demonstrated that the test plant was substantially equivalent to its comparator line.[79]While this illustrated that there were no unintended effects on the tomatoes plants metabolism, the authors advise that methods such as this are required to analyze GM plants and to "provide information absolutely crucial for a scientific solution of the problem of food safety".[80]

Methods such as GC-MS (also known as metabolic fingerprinting or profiling) and protein expression studies are not currently used to identify hazards in the Australian risk assessment process. The OGTR, in email correspondence with one of the authors, have made it clear that they consider it unlikely that hazards will be overlooked in the risk assessment process, stating that:

> The Gene Technology Regulator can seek any information deemed necessary for identifying and assessing risks. Experience from two decades of research on and commercialisation of GM crops, and

[73] Roessner U, Luedemann A, Brust D, Fiehn O, Linke T, Willmitzer L and Fernie AR, "Metabolic Profiling Allows Comprehensive Phenotyping of Genetically or Environmentally Modified Plant Systems" (2001) 13 *Plant Cell* 11.

[74] Roessner et al, n 73 at 16.

[75] Urbanczyk-Wochniak E, Luedemann A, Kopka J, Selbig J, Roessner-Tunali U, Willmitzer L and Fernie AR, "Parallel Analysis of Transcript and Metabolic Profiles: A New Approach in Systems Biology" (2003) 4 (10) *EMBO Reports* 989.

[76] Noteborn HPJM, Lommen A, Van der Jagt RC and Weseman JM, "Chemical Fingerprinting for the Evaluation of Unintended Secondary Metabolic Changes in Transgenic Food Crops" (2000) 77 *Journal of Biotechnology* 103.

[77] Corpillo et al, n 21 at 193.

[78] Sweetlove, et al, n 72.

[79] Corpillo et al, n 21 at 193.

[80] Corpillo et al, n 21 at 199.

> expanding knowledge of plant genome structure and dynamics all indicate that the GM process itself provides little potential for unexpected adverse outcomes that would not be eliminated in the variety development process. Therefore it is not expected to make metabolic fingerprinting mandatory unless there is evidence to suspect that any metabolic changes due to the genetic modification are capable of causing harm to the health and safety of people or harm to the environment.[81]

As such, there are no plans by the OGTR to incorporate procedures such as these in the risk assessment process.

Consequence of unidentified hazards

Different factors may contribute to the significance of adverse consequences which may arise as a result of hazards not being identified in the risk assessment process; namely severity, cumulative, space and reversibility.[82] The very nature of GMOs makes the consequences difficult to predict, but what is known suggests that if hazards are not identified the consequences could be of considerable magnitude.

The severity of any consequence is dependant upon the nature of the risk. In the case of an increased toxin, the concentration and nature of the toxin contribute to the severity of any consequence. If the toxin is highly toxic then the consequence could be severe, and if cumulative in nature then a potential reduction in toxicity would still result in increasing effects over time. While it is likely that acute toxicity would be detected during the short term feeding tests that are generally conducted on GM crops prior to commercialisation, this is not the case with toxins that accumulate in the environment or in living organisms and may result in long-term or cumulative toxicity issues. Given that commercialisation would result in these crops being cultivated over thousands of acres of the Australian landscape, there is potential for exposure to be widespread in the Australian environment. Likewise, the consumption of GM food will result in exposure to significant numbers of the Australian population.

Reversibility is of great importance to determining the consequence of GM crops as the ability to remove GMOs from the environment once established on a broad scale, can be extremely difficult, as the experience with Starlink corn illustrates. Starlink corn was approved for feed uses in the United States in 1998, and was subsequently planted over a relatively small area (<1%) of the United States corn acreage.[83] It was not approved for human consumption due to allergenicity concerns but was approved for cultivation for animal feed purposes. In 2000, Starlink corn was discovered by independent researchers in processed corn products on supermarket shelves in the United States.[84] A major product recall was conducted and Starlink seed was removed from the market. In 2001 the Starlink gene was discovered in commercial corn seed and was again discovered in corn products, this time in the United States and Japan. For this crop to be planted on a relatively small area and yet to have entered the global food chain illustrates how difficult it is to contain living organisms. Remediation is not simply a matter of removing the product from the market as cross-contamination can lead to its persistence in the seed supply.

A further illustration of the pervasiveness of GM crops is the recently discovered contamination of a Victorian canola shipment with a GM canola line that, although approved by the OGTR, has never been grown commercially in Australia. It is likely that imported non-GM seed was contaminated with this GM variety.[85] Given that the commercialisation of GM crops will lead to the potential for large-scale exposure and that remediation efforts are extremely difficult if post-market hazards are identified, it is imperative that a comprehensive investigation of all risk probabilities is conducted before commercial approval is granted.

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[81] OGTR, Response to questions, email correspondence with Sharon Fox (25 July 2005).

[82] OGTR, n 42, p 47.

[83] Lin W, Price GK and Allen EW, "StarLink: Impacts on the U.S. Corn Market and World Trade" (2003) 19 (4) *Agribusiness* 473.

[84] Goldman LR and Bucchini L, "Starlink Corn: A Risk Analysis" (2002) 110 (1) *Environmental Health Perspectives* 5.

[85] GRAIN, *Australia: Farmers Outraged at Canola Contamination* available online at http://www.grain.org/research/contamination.cfm?id=325 (viewed 24 April 2005).

IMPROVING RISK ASSESSMENT UNDER THE GENE TECHNOLOGY ACT (2000)

Whilst the *Gene Technology Act 2000* (Cth) and the *Gene Technology Regulations 2001* (Cth) provide little in the way of specific risk assessment methodologies, the potential risks associated with the use of gene technologies are acknowledged. Under s 49(2)(b) of the Act, the expected effects, and under Sch 4, Pt 2.2.2 of the Regulations, unintended effects, including mutation, are to be considered by the Regulator in the determination of risks to the health and safety of people or to the environment. These directives, while acknowledged by the Regulator, are not investigated using methods that have the sensitivity required to provide conclusive evidence that unintended changes to plant metabolism are identified.

Furthermore, the crop developer is required under s 7 of the Regulations to provide information "as comprehensive as existing scientific knowledge"[86] in their applications to the Regulator. If relevant data and information is not provided then the applicant must include

- a summary of known existing scientific evidence relevant to such evaluation; and
- applying that summary – an evaluation of the possible risks based on theoretical approaches, and research methods, that are generally accepted in the scientific community.[87]

The results of recent experiments demonstrating the extent of mutations induced by transformation techniques are published in peer-reviewed journals and as such represent scientific knowledge that is both available and relevant to the risk assessment of GE crops. Likewise, research using molecular profiling techniques is peer reviewed and scientifically accepted and relevant to risk assessment, with several scientists advising that these techniques are important tools for the use of GM crop risk assessment.[88] There is no evidence that this information has been considered during the assessments conducted by Australian regulators. This suggests that crop developers present only evidence that supports their findings, which in itself is an act of non-compliance under the Act, and demonstrates substantial bias. Such bias compromises the risk assessment process and should be addressed by the Regulator.

Improving the Australian risk assessment process can be achieved with relative ease and without need for amendment to the Act. Mandating the use of techniques such as GC-MS and 2-D gel electrophoresis in hazard analysis will provide a more comprehensive, sensitive and unbiased analysis of plant composition. These techniques are used by plant developers to identify genes with commercial applications and can be used to generate data that would provide a more comprehensive assessment of a GM plants metabolic state. Such analysis would demonstrate with far more certainty that a GM-plant is "substantially" equivalent to its non-GM comparator. Utilising these procedures in the hazard identification process will significantly reduce the likelihood that GM-crops have any adverse effects on the Australian public or environment.

CONCLUSION

The evidence presented here raises concerns that GE techniques may produce unintended adverse results, from theoretical concern, into the realm of the possible and indeed, even the probable. It is imperative that these issues are addressed by the Regulator, as not doing so is a failure to ensure human and environmental safety as mandated by the *Gene Technology Act 2000* (Cth) and the *Gene Technology Regulations 2001* (Cth). Claims that the Act would provide a comprehensive accountable regulatory system[89] are overstated, not due to flaws in the Act but as a direct consequence of the assumptions inherent in the risk assessment procedures. The Act and the Regulations direct the Regulator to consider the probability of unintended effects and mutation in risk considerations however, to date there is little evidence that these issues are given due attention in the decision making process.

[86] *Gene Technology Regulations 2000* (Cth), s 7(3)(a).

[87] *Gene Technology Regulations 2000* (Cth) s 7 (4)(c) and (d).

[88] Kuiper et al, n 15 at 516; Freese and Schubert, n 37 at 315: Corpillo et al, n 21 at 293.

[89] Australia, House of Representatives, n 6, p 18104 (per the Hon Dr Michael Wooldridge).

Whilst scientific evidence demonstrates that plants can be engineered to contain new genes with little change to gene expression in the plant, there is also credible evidence to suggest that genetic engineering techniques can result in unexpected, potentially hazardous changes in plant composition. The current use of a broad compositional analysis to investigate unintended changes in plant metabolism does not represent a rigorous science-based approach to risk assessment. This method of plant composition analysis is not of sufficient sensitivity to detect even large changes in plant composition and therefore its use represents a failure on behalf of the Regulator to ensure the objective of the Act is achieved. Given that hazard identification underpins the risk assessment process it is imperative that the Regulator address this issue to ensure that hazards are identified and the objective of the Act is achieved.