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REVIEW

Biosafety and risk assessment framework for selectable marker genes in transgenic crop plants: a case of the science not supporting the politics

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Abstract Selectable marker gene systems are vital for the development of transgenic crops. Since the creation of the first transgenic plants in the early 1980s and their subsequent commercialization worldwide over almost an entire decade, antibiotic and herbicide resistance selectable marker gene systems have been an integral feature of plant genetic modification. Without them, creating transgenic crops is not feasible on purely economic and practical terms. These systems allow the relatively straightforward identification and selection of plants that have stably incorporated not only the marker genes but also genes of interest, for example herbicide tolerance and pest resistance. Bacterial antibiotic resistance genes are also crucial in molecular biology manipulations in the laboratory. An unprecedented debate has accompanied the development and commercialization of transgenic crops. Divergent policies and their implementation in the European Union on one hand and the rest of the world on the other (industrialized and developing countries alike), have resulted in disputes with serious consequences on agricultural policy, world trade and food security. A lot of research effort has been directed towards the development of marker-free transformation or systems to remove selectable markers. Such research has been in a large part motivated by perceived problems with antibiotic resistance selectable markers; however, it is not justified from a safety point of view. The aim of this review is to discuss in some detail the currently available scientific evidence that overwhelmingly argues for the safety of these marker gene systems. Our conclusion, supported by numerous studies, most of which are commissioned by some of the very parties that have taken a position against the use of antibiotic selectable marker gene systems, is that there is no scientific basis to argue against the use and presence of selectable marker genes as a class in transgenic plants.

Keywords Selectable markers · Plant transformation · Antibiotic resistance · Herbicide resistance · Safety evaluation

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Introduction and Background

In the developing world 840 million people are chronically undernourished, surviving on fewer than 2,000 calories per day (Pinstrup-Andersen et al. 1999; FAO 2001; Christou and Twyman 2004). Many more people, perhaps half of the world's population in total, suffer from diseases caused by dietary deficiencies and inadequate supplies of vitamins and minerals (Graham et al. 2001). It is therefore not surprising that contemporary plant biotechnology stands to benefit huge numbers of people in the developing world to a much greater extent than consumers in the affluent West. Perhaps this is one of the reasons why well-fed Europeans, who enjoy very high living standards and quality of life, can afford to reject in the short term technologies on the basis of ideology and vested economic and political interests.

Many excellent accounts of the economic, environmental and health benefits of transgenic or genetically modified (GM) crops have been published (Huang et al. 2002; Toenniessen et al. 2003; James 2005; Ferry et al. 2006; Christou et al. 2006). In the US, the six biotechnology-derived crops planted in 2003 (canola, corn, cotton, papaya, squash and soybeans) produced an additional 5.3 billion pounds of food and fiber, and increased farm income by $1.9 billion. These biotechnology-derived crops also reduced the use of pesticides by 46.4 million pounds (Huang et al. 2005; Christou et al., 2006). The current status of Bt rice, which is expected to be commercially released in China in the near future is reviewed by High et al. (2004). Farm surveys of randomly selected households cultivating insect resistant transgenic varieties demonstrate that when compared with households cultivating non-transgenic rice, small and poor farm households benefit from adopting transgenic rice by both higher crop yields and reduced use of pesticides, which also contribute to improved health. For cotton, the key documented benefits are a 70% reduction in insecticide applications in Bt cotton fields in India, resulting in a saving of up to US$30 per hectare in insecticide costs, with an increase of 80–87% in yield of harvested cotton (Huang et al. 2002) and a dramatic reduction in pesticide applications in Bt cotton fields in China. The same survey revealed that the percentage of farmers with pesticide poisoning was reduced from 22% to 4.7% (Brookes and Barfoot 2005; http://www.ncfap.org/40CaseStudies.htm; http://www.ncfap.org/Europe.htm).

The objective of this review is to focus on key aspects of the science underpinning the evaluation and risk assessment for the safety of transgenic crops that contain selectable marker genes. We will emphasize mostly antibiotic and to a lesser extend herbicide resistance genes only, as these are the most widely used markers and therefore have been the subject of much attention. We argue for broader acceptance of selectable marker genes in general based on safety data; there does not appear to be a major issue at present with herbicide tolerance genes as targets for elimination in transgenic food or feed crops. An excellent and comprehensive review on selectable marker genes in transgenic plants has been published (Miki and McHugh 2004). This review provides an in depth discussion of approximately 50 such marker genes, and sets the background for our review which focuses exclusively on marker gene systems used for transgenic crops that are currently on the market.

Regulatory practitioners often face the reality that governments apply non-scientific standards to their decision-making. This unfortunate fact places severe limits on the molecular tools for creating transgenic food and feed crops that require global regulatory approvals. A major objective of this review is to address this dichotomy, and we acknowledge that the arguments we put forward here focus solely on the science-based safety rationale for continued use of selectable marker genes.

We start by reviewing the scientific principles behind the regulations for the safety assessment for transgenic crops. These are generic in nature and apply to all foreign genes, including selectable markers. We focus on a number of key aspects of the assessment process namely: whether heterologous proteins that detoxify antibiotics, or herbicides used together with selectable marker genes expressed in crop plants, are inherently toxic or allergenic; the likelihood and consequences of horizontal gene flow to microorganisms; vertical gene flow through pollen transfer and its consequences; and the digestive fate of recombinant marker DNA and proteins. We particularly discuss transgenic crops used in animal feed, as the overwhelming majority of such crops currently grown worldwide are used for this purpose. In the course of this analysis we discuss additional

relevant issues such as the concept of substantial equivalence as a cornerstone for a meaningful and practical risk assessment evaluation.

General principles in the safety assessment of transgenic crops

All transgenic plants are required to undergo thorough and rigorous safety and risk assessments before commercialization. A risk assessment consists of hazard identification, hazard characterization, exposure assessment and risk characterization (EC 2002; Codex Alimentarius 2001). Regulatory justifications for these assessments differ between countries, although they require similar tests. In the US for example, the process is based on the determination of substantial equivalence, whereas Europe has passed regulations based more on certification of the process rather than of the product, and Canada regulates the product itself, irrespective of the process used to generate it. In the US and Europe no such formal assessment is required for products obtained with conventional methods. In Europe transgenic plants are subject to special regulations including a horizontal directive (EC 2001) that commences from research and development through release onto the market, and vertical rules governing specific areas including food safety and traceability (EU Regulation 1829/2003).

In 2003, the Codex Alimentarius published guidelines for the conduct of food safety assessment of foods derived from recombinant-DNA plants (CAC/GL 45-2003) in which it specifically commented on the use of antibiotic resistance marker genes (Section 5—Other Considerations, page 8). Codex also published a companion document (CAC/GL 46-2003) on food safety assessment of foods derived from recombinant-DNA microorganisms. One of the recommendations stipulates that: ''Alternative transformation technologies that do not result in antibiotic resistance marker genes in foods should be used in the future development of recombinant-DNA plants, where such technologies are available and demonstrated to be safe.'' This is an unfortunate general statement since the Codex Alimentarius is recognized as the international standards setting organization for food safety and its pronouncements carry substantial weight in international trade matters under the World Trade Organization. Such statements mostly harm developers of products and technologies in the developing world where individual laboratories might not have the luxury of carrying out cosmetic research and development that most likely is covered by intellectual property.

The risk assessment process for transgenic plants consists of two steps: (i) a comparative analysis (substantial equivalence) to identify potential differences with their non-engineered counterpart(s), followed by (ii) an assessment of the environmental and food/feed safety or nutritional impact of any identified differences. The risk assessment process requires clear identification of any differences between the transgenic and non-transgenic crop(s), including management and usage, and is meant to focus on the significance and implications of any differences (EFSA 2004a).

Substantial equivalence

The concept of substantial equivalence is based on comparisons of transgenic plants and products with their non-transgenic counterparts. If a new plant/food product is found to be substantially equivalent in composition and nutritional characteristics to an existing plant/food product, it can be regarded as being as safe as the conventional food; however, in practice extensive safety testing still gets done even for those transgenic crops that are considered substantially equivalent (FDA 1992; OECD 1993; Maryanski 1995). Evaluation of substantial equivalence includes considerations of the characteristics of the transgene and its likely effects within the host; measurements of protein, fat and starch content; amino acid composition and vitamin and mineral equivalency, together with levels of known allergens and other potentially toxic components. Such plants or products can either be substantially equivalent to an existing counterpart, substantially equivalent except for defined differences (on which further safety assessments would then be focused on), or non-equivalent, which would imply that more-extensive safety testing might be necessary (FAO/WHO 1996; Royal Society 2002). However, safety testing might be more challenging as whole foods cannot be tested with the high-dose strategy that is currently used for single chemicals to increase the sensitivity in detecting toxic endpoints (MacKenzie 1999; Royal

Society of Canada 2001). Substantial equivalence has therefore been suggested by some as the starting point of the safety assessment (Kok and Kuiper 2003). Even though this has some merit, it is becoming clearer that further assessment requirements proposed in certain quarters make little practical sense and are not justified in view of the collective knowledge accumulated on the subject over the past two decades. An issue arises in that risk factors have generally not been established for plant varieties developed using conventional technologies and it is for this reason that limited baseline information exists about the environmental and other risks associated with their introduction. Perhaps this should be an area of high-priority research, i.e., to build databases that will facilitate the evaluation of substantial equivalence, rather than investing heavily in the development of even more-sensitive analytical capabilities, which thus far have given regulators few additional tools for a rational safety assessment in return. The International Life Sciences Institute has established such a database, which is available online at www.cropcomposition.org.

It is important to recognize that the nutritional composition of crops is also influenced by environmental and other factors, which include time of harvesting, post-harvest storage conditions and processing. In a recently published study, the substantial equivalence of field-grown transgenic wheat was evaluated using metabolic profiling. The conclusion of this study was that the environment affects the metabolome, and any differences between transgenic lines and controls are generally within the same range as the differences observed between the control lines grown on different sites and in different years (Baker et al. 2006). Baudo et al. (2006) determined that transgenesis had less impact on the transcriptome of wheat grain than conventional breeding. Differences observed in gene expression in endosperm and leaves between conventionally bred materials were much larger in comparison to the differences between transgenic wheat plants expressing high-molecular-weight glutenin subunits and the *bar* marker gene and untransformed lines exhibiting the same complements of gluten subunits. It is not unreasonable to expect that, if the same stringent testing criteria as those required for transgenic crops were to be applied to crops produced conventionally, many such crops with a history of safe use may be shown to be unsafe (Pastorello et al. 1998; MRC 2000). Toxins (for example carcinogens such as mycotoxins) can be produced by fungi before harvesting or during storage (reviewed in Halford and Shewry 2000) and may thus render the crop unsafe for consumption. As outlined above, the introduction of new types and varieties of food crops produced by conventional breeding requires no specific testing for the presence of allergens and toxins, although genes may have been introduced from exotic varieties or related wild species. The concept of substantial equivalence requires the comparison of the GM crop to its appropriate safe comparator; however with the comparator's safety identity unknown in many cases, this approach may frequently be confounded. Thus, GM foods are at least as safe as foods derived from non-GM varieties.

Consensus issues in the safety assessment process

OECD countries and the United Nations Food and Agriculture Organisation (FAO)/World Health Organisation (WHO) through a process of expert consultations have arrived at a consensus on the specific safety issues that should be considered when evaluating a novel food (OECD 2000). These include: (i) description of the host organism that has been modified, including information on nutrient composition, known anti-nutrients, toxicants and allergenic potential, and any significant changes in these that may result from normal processing; (ii) description of the donor organism, including any known associated toxicities and allergenicities, and the introduced gene(s); the gene products should pose no significant risks to human health or non-target organisms, and none of the inserted sequences should be known to have any pathogenic or harmful characteristics; (iii) molecular characterization of the genetic modification, including a description of the modification process and the stability of the introduced trait; a detailed description of the molecular characteristics of the transgenic plant is required in order to demonstrate that the developer has critically analyzed the plant and its products, including all novel genes and novel proteins; the inheritance, stability and expression of each introduced trait that is functional in the transformed plant must be demonstrated over a number of generations; (iv) identification of the primary and any secondary gene products, including

a description of the characteristics of the inserted gene; (v) evaluation of the safety of expected novel substances in the food, including an evaluation of any toxins produced directly by the modification; (vi) assessment of the novel food-potential allergenicity; and (vii) evaluation of unintended effects on food composition, including: assessment of changes in the concentration of nutrients or naturally occurring toxicants, identification of anti-nutrient compounds that are significantly altered in novel foods, and evaluation of the safety of compounds that show a significantly altered concentration.

Selectable marker systems in the development of transgenic crops

The creation of transgenic plants is a very long, labor-intensive, costly and inherently inefficient process. This is particularly the case when one moves away from model laboratory species to the more important agricultural crops (Stoger et al. 2002; Christou et al. 1991; Christou 1996; McCabe et al. 1988). The use of selectable marker gene systems facilitates the transformation process and allows the relatively straightforward recovery of transgenic crop plants. Without them, the few plant cells that take up and stably integrate the foreign DNA would simply be lost in an ocean of wild-type cells, which would certainly overgrow these transformed cells in the absence of an effective selection against them. There are two major classes of such genes: antibiotic and herbicide resistance genes. Antibiotic resistance genes are used in two important phases of transgenic plant production: (i) pre-plant transformation to select bacteria during routine molecular biology operations to manipulate transgenes and create expression vectors, and (ii) during the transformation process itself, to select cells and plants that have stably integrated introduced transgenes (selectable marker and gene(s) of interest).

The key issues

Issues frequently raised in this context with respect to antibiotic resistance genes include: (i) effects on the therapeutic efficacy of clinically used antibiotics, i.e., concerns that antibiotic resistance gene products in transgenic crops or products might render clinically important therapeutic antibiotics ineffective; (ii) potential for horizontal gene transfer, i.e., concerns about the potential transfer of the antibiotic resistance marker genes to intestinal and soil microorganisms.

For herbicide resistance genes, the issues are somewhat different: (i) gene flow—in by which new genes can spread by normal out-crossing to wild or weedy relatives of the engineered crop; this becomes an issue only if the new trait(s) confers a fitness advantage after it becomes stably introgressed into the recipient relative and the recipient relative itself becomes invasive; (ii) weediness—the potential for a crop or its sexually compatible wild relatives to become established and to persist and spread into new habitats as a result of newly introduced genes; (iii) toxicity and allergenicity—an issue associated with human health and the safety of novel foods and potential negative effects on non-target organisms.

Origin of the commonly used selectable marker gene systems in commercialized transgenic crop plants

All commonly used antibiotic and a number of herbicide resistance genes are derived from bacteria that are ubiquitous in nature. Humans, plants and animals are exposed to them daily through direct contact and diet. The *nptII* (*aph(3′)II*) kanamycin resistance gene originates from transposon Tn5 of *Escherichia coli* K12 (Garfinkel et al. 1981). Kanamycin, the antibiotic which *nptII* inactivates, is rarely used in medicine because of its considerable side effects and its use has been superseded by more-effective aminoglycoside antibiotics that are not substrates for *aph(3′)II* (Nap et al. 1992). Twenty to forty percent of naturally occurring bacteria in animal or human digestive tracts are already resistant to kanamycin (EFB 2001).

The hygromycin resistance (*hph*) gene originates from *Escherichia coli* W677. Two major genes encoding the protein have been characterized, one from *Streptomyces hygroscopicus* (Leboul and Davies 1982; Malpartida et al. 1983) and the second from *E. coli* (Rao et al. 1983; Kuhstoss and Rao 1983) and *Klebsiella pneumoniae* (Gritz and Davis 1983). For the most part, the *hph* gene used in transgenic plants is derived from *E. coli*. Hygromycin is not in human clinical use.

The streptomycin resistance *aadA* gene originates from *E. coli* and is ubiquitous among Gram-negative bacteria (Shaw et al. 1993). It has been cloned from several transposons, including the multi-resistance transposon Tn1331 in *Klebsiella pneumoniae* (Tomalsky and Crosa 1987). Plasmids carrying streptomycin resistance genes are very common and can be found at high frequency in naturally occurring bacterial populations (Shaw et al. 1993). Streptomycin has been replaced by newer, more effective antibiotics for human applications. The chloramphenicol resistance *CmR* gene is also an *E. coli* gene (Proctor and Rownd, 1982). Microorganisms resistant to chloramphenicol are widely distributed in nature (Murray and Shaw 1997). The ampicillin resistance gene, amp^r, has also been isolated from *E. coli*. The original plasmid with its transposon Tn3 and the beta-lactamase gene (amp^r, $bla_{(TEM-1)}$) were originally isolated from a hospital bacterium isolate TEM in 1963 (Jacoby and Medeiros 1991). Ampicillin resistant bacteria are normally found in the human intestine and are also present in high concentrations in broilers, cattle and pigs (DANMAP 2001). Ampicillin resistance occurs in up to 10% of bacteria in the environment (EFB 2001).

Bacteria have to compete in nature with other microorganisms for their very survival, including other bacteria and fungi. They have developed very sophisticated mechanisms to produce antibiotics to eliminate competitors and assure their own survival. Simultaneously with their ability to produce such compounds, bacteria also need to have a defense against the compounds they themselves produce to eliminate their competitors, in the form of resistance genes. Over time, target bacteria counter the effects of antibiotics produced by other bacteria through resistance mechanisms. Rather than developing their own resistance mechanisms, bacteria frequently acquire antibiotic resistance genes that are already present in the bacterial pool surrounding them (EFB 2001). Bacteria have well-developed mechanisms to accomplish this (Bennett et al. 2004). The presence of an antibiotic confers an advantage to a resistant bacterium and as a consequence, the development of resistance and spread increases (EFB 2001).

Bennett et al. (2004) questioned whether the transfer of a number of commonly used antibiotic resistance genes in plant molecular biology, if it were to take place in bacteria, would pose a threat to public health. The authors first provided a very comprehensive and in-depth analysis based on the current knowledge of bacterial DNA transfer mechanisms and recombination systems. Following this analysis and after concluding that such transfer is highly unlikely, at best, under natural conditions, they examined whether any potential transfer would have any consequences. They focused on the bla_{TEM}, *aph*(*3′*) and *aadA* genes. These genes are very common in many bacteria in nature and also among the *Enterobacteriaceae*, and are located on mobile genetic elements that have moved extensively between DNA molecules and bacterial cells. The authors correctly point out that this gene mobility has ‘‘already severely compromised clinical use of antibiotics to which resistance is conferred’’. They therefore conclude that ‘‘the argument that occasional transfer of these particular resistance genes from transgenic plants to bacteria would pose an unacceptable risk to human or animal health has little substance’’.

In a recent study, D’Costa et al. (2006) surveyed a wide range of spore-forming soil-dwelling bacteria from urban, agricultural and forest samples. A library of 480 strains was constructed and screened against 21 clinically relevant antibiotics covering a wide range of structural complexity. The antibiotics encompassed all major bacterial targets and included drugs that have been on the market for decades, as well as recently approved compounds. Without exception, every strain in the library was found to be multi-drug resistant to 7–8 antibiotics on average, with two strains resistant to 15 of 21 drugs. The authors reported reproducible resistance to most of the antibiotics, regardless of origin and almost 200 different resistance profiles were seen, exemplifying the immense genetic and phenotypic diversity of the collection of bacteria. The authors acknowledge that their study does not provide evidence for the direct transfer of resistance elements from the soil resistome to pathogenic bacteria; however, it does demonstrate an unprecedented density and concentration of environmental antibiotic resistance. The level and diversity of resistance that their studies uncovered is underestimated because only spore-forming bacteria were studied and these represent only a fraction of soil-dwelling bacteria.

The European Food Safety Authority (EFSA) adopted an opinion on many of the issues surrounding the use of antibiotic selectable markers in transgenic

plants (EFSA 2004b). The opinion is relevant in the context of Directive 2001/18/EC (EC 2001) which is targeted towards ''identification and phasing out antibiotic resistance marker genes in genetically modified organisms (GMOs) which **may have adverse effects on human health and the environment**'' (emphasis added). It is interesting to note that the EU directive does not mention, in any way, the elimination of all antibiotic selectable marker genes from transgenic plants. Rather it focuses on those that may have adverse effects. The EFSA considered the vast body of evidence in the scientific literature and concluded that the antibiotic markers that are now present in transgenic plants on the market present no inherent risk to human health, veterinary medicine or the environment, because of the wide prevalence and high frequency of these genes in natural bacterial populations. The conclusion by the EFSA was that, even if there were gene transfer between transgenic plants and bacteria, this event would be completely inconsequential. An illustrative example is provided by an analysis focusing on the *nptII* gene. The rate of DNA transfer between bacteria under optimal conditions in nature is in the range of 10^{-2} to 10^{-5}. The estimated frequency of uptake of the same gene from transgenic plants to bacteria under optimal conditions is 10^{-17}, an infinitesimal number. The *nptII* gene corresponds to only 0.00004% of the total maize genome and thus would have to compete with the rest of the DNA for uptake by the bacterium. The availability of free transgenic plant-derived DNA in the rumen or the gastro-intestinal tract is further limited due to rapid digestion by pancreatic fluids and acid saliva. The contribution of the *nptII* gene from transgenic plants to the overall pool of kanamycin resistant bacteria appears to be insignificant when compared to the large pool of genes providing resistance towards kanamycin that already exists in bacteria. Therefore, a bacterium is several orders of magnitude more likely to acquire a resistance gene from another bacterium, rather than from the DNA of the transgenic crop (EFB 2001).

Transgenic crops used as animal feed

Feeds derived from transgenic crops comprise a large percentage of the daily diet of animals, often for the complete lifespan of the animal. There are three key issues in developing a unified approach to the safety assessment of transgenic crops used as animal feed (reviewed in Aumaitre et al. 2002):

(i) Substantial equivalence is important for the nutritionist since it provides the basis for concluding on the absence/presence of any unintended effects introduced by the genetic modification process; the key issues for animal nutritionists are: are nutritional equivalence and substantial equivalence synonymous, or is one evidence of the other? Should nutritional parameters always be considered in establishing substantial equivalence for a transgenic plant used as an animal feedstuff?

(ii) The safety of the introduced gene products for humans and animals requires acute toxicity studies on laboratory animals; feeding of the whole plant (or part thereof) to farm animals at normal dietary levels during safety assessments, is open to debate (OECD 2000). There is the need to extrapolate from the responses induced in animals, to evaluate the likely impacts on human health. Animals can be used for the identification of acute toxicity, by administration of a large single dose followed by 14 days of observation. Animals are thus exposed to high doses of purified gene product that exceed human exposure levels by several orders of magnitude (Entransfood 2004). Some feel that whole-food testing should be allowed in order to answer the question of whether unintended adverse effects (e.g., insertion of the new gene that might increase levels of endogenous protein expression) might occur. Others however, feel that tests on the introduced gene product, which represents only a small fraction of the total diet, cannot easily be performed when the ingredients to be tested normally account for 20 to 90% of the diet. The detection of potential adverse effects and relating these conclusively to an individual characteristic of the food (feed) can therefore be extremely difficult (FAO/WHO 2000; Codex 2001).

(iii) The assessment of the safety of products derived from animals raised on feeds of transgenic origin is not specifically addressed in legislation. This fact in no way reflects a lack of oversight by the regulators. The

> expression of heterologous proteins or the absorbance of newly expressed proteins by animal products is considered to be unlikely (CAST 2006). It is therefore not considered necessary to routinely test for the presence of introduced genes or their products unless their characteristics suggest cause for concern (EC 2003). Data dealing with the effect of GM crops in animal nutrition on the quality of end product are rather scarce in the literature, and no country currently requires the labeling of eggs, meat and milk from animals fed with GM feed.

The safety assessment of a novel livestock feed considers the molecular, compositional, toxicological and nutritional characteristics of the novel feed compared to its conventional counterpart. Safety considerations focus on the animal eating the feed, consumption of the animal product(s), worker safety and any other environmental aspects that might arise from use of the feed. Indirect effects of the consumption of feeds include: the likelihood that transgenes or recombinant proteins will be transferred to, and accumulate in, food products (milk, meat, eggs, and fish); and whether the consumption of animal products will lead to adverse health effects in humans. These issues are addressed by considering the normal digestive fate of DNA and proteins present in all foods, the digestibility of new proteins expressed in transgenic plants, and investigating the occurrence of transgenic DNA and proteins in food products of animal origin (summarized in MacKenzie and McLean 2002). Results of assessments focusing on these and other issues are discussed in detail in subsequent sections.

In order to determine the nutritional significance of a transgenic crop used as animal feed, substantial equivalence studies on the chemical components such as crude protein, fat or other extract, fiber, starch, amino acids, fatty acids, ash and sugar, depending on the nature of the plant and its major components, are carried out (Aumaitre et al. 2002). Other analyses are based on determining the levels of anti-nutrients. Nutritional data (for example from oilseed obtained from the transgenic crops used for producing meals for farm animals) can be also important as they can affect the fatty-acid composition of the animal tissue (Aumaitre et al. 2002; FSANZ 2003). Feeding value trials should be carried out with representative target animals on a case-by-case basis when substantial equivalence is not demonstrated in a GM crop. It is also recommended that comparative growth studies are conducted with a fast-growing livestock species such as the broiler chick, where the growth rate is particularly sensitive to the presence of toxic elements in their feed (EC 2003). However some feeding value trials have been carried out purely to provide additional data to confirm the safety of the GM crop feedstuff, even when the crop was shown to be substantially equivalent to its counterpart. Studies have been published on both substantial equivalence and livestock feeding with the transgenic crops used as feed, with specific reference to selectable marker genes (Table 1). It is important to recognize that these crops contain genes other than marker genes, and consequently the assessment encompasses more than the marker genes. All these studies have concluded that the transgenic crops were substantially equivalent to their conventional counterparts, and had no deleterious effect on animal health or performance, when animals were fed with these crops.

Animal feeding trials are sometimes prescribed to confirm that the digestibility of transgenic crops used in animal feed is not adversely affected. Genes and recombinant proteins coding for insect resistance and herbicide tolerance have been fed without any adverse effects to rodents in acute toxicity tests at very high doses, typically in excess of 1,000 times the level at which humans or animals would consume these (Harrison et al. 1996).

Several reviews summarizing the results of studies in many species have concluded that animals fed with GM crops show no differences in performance compared to animals consuming non-GM varieties (Aumaitre et al. 2002; Clark and Ipharraguerre 2001; Flachowsky and Aulrich 2001; Wu 2006). Additionally, no studies have shown deleterious effects on livestock performance resulting from the consumption of commercially grown GM crops. A benefit resulting from feeding of GM crops has been noted in the case of insect resistant corn. Molds that often grow at the site of insect damage can produce several fungal toxins, including the deadly fumonisin. Therefore, since insect resistant corn sustains less insect damage, it is less susceptible to contamination by these toxins and the resulting corn is safer for both livestock and human consumption (Munkvold et al.

Table 1 Studies on substantial equivalence and feeding value of GM crops containing selectable marker genes (livestock)

Selectable marker	GM crop	Target livestock			
		Pigs	Poultry	Ruminants	Others[d]
neo	Maize	Weber et al. (2000) [a,b]	Taylor et al. (2003)[a,b]		
		Hyun et al. (2005)[a,b]			
	Cotton			Castillo et al. (2004)[a,b]	
				Hamilton et al. (2004)[a,b]	
	Potato				Sanhoty et al. (2004)[a,b]
pat	Maize	Aulrich et al. (2002)[a,b,c]	Aulrich et al. (2002)[a,b,c]	Aulrich et al. (2002)[a,b,c]	
		Bohme et al. (2001)[a,b]	Brake et al. (2003)[a,b]	Phipps et al. (2005)[a,b]	
				Bohme et al. (2001)[a,b]	
	Canola		FSANZ (2003)[a,b]		FSANZ (2003)[a,b]
	Sugar beet	Aulrich et al. (2002)[a,b,c]	Aulrich et al. (2002)[a,b,c]	Aulrich et al. (2002)[a,b,c]	
		Bohme et al. (2001)[a,b]	Bohme et al. (2001)[a,b]		
	Rice	Cromwell et al. (2005)[a,b]			
epsps	Soybean	Cromwell et al. (2002)[a,b]	Hammond et al. (1996)[b]	Phipps et al. (2002)[b]	Padgette et al. (1996b)[a]
		Jennings et al. (2003)[b]	Clark and Ipharraguerre (2001)[a,b,c]	Hammond et al. (1996)[b]	Hammond et al. (1996)[b]
				Clark and Ipharraguerre (2001)[a,b,c]	Sanden et al. (2006)[b]
	Maize		Taylor et al. (2005)[b]	Grant et al. (2003)[b]	
			Sidhu et al. (2000)[a,b]	Donkin et al. (2003)[b]	
			Clark and Ipharraguerre (2001)[a,b,c]	Ipharraguerre et al. (2003)[a,b]	
				Clark and Ipharraguerre (2001)[a,b,c]	
	Cotton			Castillo et al. (2004)[a,b]	Nida et al. (1996)[a]
					Bertrand et al. (2005)[a]
	Wheat		Kan and Hartnell (2004)[b]		
	Sugar beet			Hartnell et al. (2005)[b]	
	Canola	Sharma et al. (2006)[b]	Taylor et al. (2004)[b]	Standford et al. (2003)[b]	
				Sharma et al. (2006)[b]	

[a] Substantial equivalence

[b] Feeding value

[c] Review

[d] Others, including studies of substantial equivalence and feeding value in other animals (rats, mice, fish)

1997; Munkvold et al. 1999; Flachowsky and Aulrich 2002). Animals digest proteins from transgenic crops in the same way as they digest proteins from non-transgenic crops. Dietary DNA and proteins are degraded during the digestive process. A number of studies indicate that introduced DNA or proteins from transgenic crops are not detected in milk, meat, or eggs from animals that consume feed components derived from these crops (Einspanier et al. 2001; Flachowsky and Aulrich 2002; Phipps et al. 2002; Phipps et al. 2003). Plant DNA has been detected in the muscle, liver, spleen and kidneys of broilers and layers, although not in eggs. However, no fragments of transgenic DNA or its expressed protein have been found to date in poultry meat or eggs or in any other animal tissues examined (Chesson and Flachowsky et al. 2003).

Experiments performed by Chambers et al. (2002) demonstrated that the bla_{TEM} marker gene (present in a transgenic maize product used as animal feed) was very unlikely to be transformed into bacteria found in the lower gut flora of chickens, for ampicillin resistance. The conclusion from this study was that animals fed with transgenic feed are unlikely to be vectors for horizontal gene transfer from transgenic plant material to the gut microflora, including significant human pathogens.

Digestive fate of recombinant DNA and proteins

DNA present in any food can find its way into cells of humans/animals that consume the food/feed at some low frequency. In the unlikely event that the DNA is recombined into a host chromosome, the probability that it will exert any biological effect in the whole organism is even more remote. Even in this highly unlikely hypothetical scenario, there is no obvious mechanism through which a mammalian or human cell with altered biological properties due to foreign DNA uptake could transmit this effect to other cells, or affect the germ line of the host organism (The Royal Society 2002).

The digestive fate of new proteins introduced into transgenic crops, have been evaluated by examining their in vitro digestibility in simulated gastric and intestinal fluids (Entransfood 2004; Sharma et al. 2004; Bertrand et al. 2005; Fuchs et al. 1993; Wehrman et al. 1996), and by testing for the occurrence of these proteins (or their fragments) in food products from livestock animals (Aulrich et al. 2002; Aumaitre 2004; FASS 2006; Jennings et al. 2003; Phipps et al. 2005; Faust and Miller 1997; Ash et al. 2000). The outcome of these studies is that novel proteins expressed in transgenic crops used in livestock feed are rapidly degraded by the acid and the enzymes in the stomach and in the intestine, into single amino acids and short peptides. This means that they are not available as whole protein and they are not detectable in food products derived from these animals (MacKenzie and McLean 2002). Several studies have been conducted and these failed to detect such recombinant proteins in poultry (muscle, liver, egg whites, egg yolks, mid gut tissue) or ruminants (milk, muscle, spleen). Consistent with these results, a sensitive ELISA test did not detect the CP4 EPSPS protein in whole eggs, egg whites, liver, or feces from laying hens fed a diet containing glyphosate-tolerant soybean over their seven-week productive life (Ash et al. 2003). Cows that were fed on a diet containing one of two glufosinate herbicide tolerant and insect resistant corn hybrids, did not show any adverse effects and produced the same volume and composition of milk as cows fed on a control diet (Faust and Miller 1997; CAST 2006). There was no evidence for the presence of the transgenic proteins, Cry1Ab or phosphinothricin-N-acetyltransferase (PAT), in the milk.

Sharma et al. (2006) investigated the passage of transgenic DNA through the ruminant and nonruminant (monogastric) digestive tracts using lambs and pigs fed with transgenic glyphosate tolerant canola meal, under conditions typical of commercial livestock production. Native and transgenic DNA exhibited similar stability and persistence in the gastrointestinal tract. Fragments of both DNA types were detected in intestinal digesta, available for uptake into animal tissue. The techniques used enabled demonstration of uptake of low-copy endogenous and transgenic DNA fragments in gastrointestinal tract tissues of both lambs and pigs, and for the first time, uptake of transgene fragments into visceral tissue (kidney, liver) in pigs was also observed. The study suggests that the likelihood of uptake of transgenic plant DNA into organ tissues of ruminant or monogastric animals, although possible, is low. These authors further state that there was no evidence

to suggest that recombinant DNA would be processed in the gut in any manner different from endogenous feed-ingested genetic material. Such data therefore add to the growing body of evidence supporting the conclusion that it is highly unlikely that intestinal bacteria will acquire genes from plants, including genes that confer resistance to antibiotics. It is important to reiterate that, even if intestinal bacteria were able to acquire such antibiotic resistance genes, the consequences would be insignificant.

Studies using sensitive polymerase chain reaction (PCR) amplification techniques did not detect even small fragments of *cp4 epsps* gene in products obtained from animals fed diets containing herbicide tolerant soybean. Transgenic DNA could not be detected in milk from cows receiving up to 26.1% of their diet as herbicide (glyphosate)-tolerant soybean meal (Phipps et al. 2002). The transgene was detected in the solid phase of rumen and duodenal digesta from lactating dairy cows fed GM soybean meal (*cp4 epsps* gene for Monsanto Roundup Ready Soybean event GTS 40-3-2), but it was not detected in liquid phases of ruminal and duodenal digesta, milk, faeces or blood indicating the rapid degradation of DNA in these environments (Phipps et al. 2003).

Barriers to the transfer and expression of transgenes in other organisms

Gene transfer of exogenous integrated DNA in plant genomes to other organisms is highly unlikely to occur because of a multitude of biological barriers that exist in nature. Free DNA is rapidly degraded by nucleases and even if some were to survive digestion and were transferred, integrated and expressed in an unintended mammalian/human host, epithelial cells are short lived and would slough off to be replaced by untransformed cells (McAllan 1980, 1982). There is no recognized mechanism through which foreign genes, including antibiotic resistance genes integrated in plants, could be transferred from the plant genome to gut microorganisms, even if they could survive digestion. Several deliberate attempts have been made to transform naturally competent bacteria with transgenic plant DNA from different plants, but no gene transfer has been found to occur from genetically modified plants to soil bacteria despite the detection of the transgene in the soil (de Vries et al. 2003; Dale et al. 2002; Kay et al. 2002; Gebhard and Smalla 1998, 1999; Nielsen et al. 1998).

Bennett et al. (2004) considered bacterial DNA transfer systems and mechanisms of recombination that jointly might transfer antibiotic resistance genes from transgenic plants to bacteria. The processes of bacterial conjugation, transduction and transformation are the only known mechanisms through which bacteria might take up foreign DNA. In combination with homologous recombination, transposition, site-specific recombination and DNA repair, these three DNA transfer systems provide the only theoretical mechanisms for such transfer. The authors categorically state that ‘‘it is not possible to identify a credible scenario whereby new drug-resistant bacteria would be created’’. Furthermore, the potential transfer of genes from transgenic plants to microorganisms is strongly restricted by biological and physical barriers (Bertolla and Simonet 1999). These barriers include: degradation by acid and nucleases in the stomach and intestines (Jonas et al. 2001); restriction and modification systems in bacteria that destroy foreign DNA that enters the cell (Frank 1994); absence of homologous ends for efficient integration into the bacterial genome (Fink and Moran, 2005) and lack of selective pressure. In addition, when any DNA (including antibiotic resistance genes) is integrated into the plant genome, the codon usage may have been altered for more-efficient expression in the plant and the gene may have picked up methylation patterns of the plant. If this DNA is now taken up by a bacterium, it would be recognized as foreign and degraded by the microorganism's restriction endonucleases, thus making integration into the bacterial genome and subsequent expression even more unlikely.

When a transgene is introduced into a plant cell, it will not be expressed unless the appropriate promoter and terminator sequences accompany the transgene. Additionally, it is common practice to include introns in genetic constructs for enhanced levels of transgene expression in plants, in particular cereals crops such as rice, wheat, corn, barley, etc. Thus, these genetic elements when introduced together will render the expression of an integrated transgene nonfunctional and thus useless in bacteria, as these elements are specific for expression in plants.

A number of studies have looked for evidence for the transfer of antibiotic resistance genes from

transgenic plants to bacteria. No evidence has been documented to demonstrate such transfer, at least from nuclear transformants (Smalla et al. 2000; Syvanen 1999). In the laboratory and under highly favorable conditions that are unlikely to be reproduced naturally, transfer of antibiotic resistance genes from transplastomic plants was reported (Kay et al. 2002; Tepfer et al. 2003). This however, is not surprising if one considers the prokaryotic origin of plant chloroplasts. Therefore, even though such transfer was demonstrated, it is inconsequential in practical terms, as the genes involved are already widely distributed in natural bacterial populations. In addition, the FDA takes the following into consideration when evaluating the use of antibiotic resistance marker genes in crops: (i) is the antibiotic an important medication; (ii) is it frequently used; (iii) is it orally administered; (iv) is it unique; (v) will there be selective pressure for transformation to take place; and (vi) what is the level of resistance to the antibiotic present in bacterial populations in nature (FDA/CFSAN 1998)? Marker genes that encode resistance to clinically important antibiotics should not be and are not used in transgenic plants. Based on these considerations, the kanamycin resistance gene *npt*II used in transgenic crops was approved by the FDA and has since then, become the most common antibiotic resistance gene occurring in commercial transgenic crops (FDA 1998).

Case studies

Neomycin phosphotransferase II [NPTII or APH(3′)II]

An acute gavage mouse study confirmed that the NPTII protein (conferring resistance to kanamycin) caused no deleterious effects when administered by gavage at a cumulative target dosage of up to 5,000 mg/kg of body weight. This dosage correlates to at least a million-fold safety factor relative to the average daily consumption of potato or tomato, assuming all the potatoes or tomatoes consumed contained the NPTII protein. In this study, it was also demonstrated that the NPTII protein is highly sensitive to in vitro enzymatic hydrolysis. These results along with previously published information, confirm that ingestion of genetically engineered plants expressing the NPTII protein, poses no safety concerns (Fuchs et al. 1993). Additionally, Calgene Inc. conducted a thorough review and analysis on the use of the *kan^r* gene and gene product, aminoglycoside 3′-phosphotransferase II (APH(3′)II), for use as a selectable marker in FLAVR SAVR™ tomatoes, in BXN™ cotton and oil-modified rapeseed (Food Additive Petition 3A4364, 1993; Redenbaugh et al. 1994). The data in the review supports the conclusions that NPTII protein is not a toxin or allergen; that the *kan^r* gene is highly unlikely to move from the plant genome into microorganisms via horizontal gene transfer; that if such transfer were to occur, the impact would be minimal; and that NPTII protein in transgenic plants will not compromise antibiotic use in humans or animals (Redenbaugh et al. 1994). The *nptII* gene product was similarly determined to be nontoxic for human or animal consumption (Nap et al. 1992; Flavell et al. 1992). This gene and its gene products when expressed in tomato plants, underwent similar toxicological tests to determine its safety: heating steps in commercial processing and cooking methods denatured and inactivated the gene product; the tomato DNA ingested by humans was degraded in the stomach and small intestine; and in processed tomatoes, the pH is 4.6 or lower, which is far below the pH optimum of NPTII protein (reviewed in Food Additive Petition 1993). Additionally, human in vivo toxicity studies demonstrated that the kanamycin resistance gene and gene product had no adverse effects on human health (Kasid et al. 1990; Blaese et al. 1990; UNDP 2001; Gay and Gillespie 2005).

5-enolpyruvylshikimate-3-phosphate synthase (EPSPS)

The EPSPS enzyme is naturally present in foods derived from plant and microbial sources. The safety of consuming the CP4 EPSPS protein was established based on its similarity to the structure and function of the naturally occurring plant EPSPS enzymes; the lack of toxicity or allergenicity of EPSPS proteins from plants, bacteria and fungi; and by direct laboratory studies on the toxicity and characteristics of the CP4 EPSPS protein (Brake and Evenson 2004; Burks and Fuchs 1995; Hammond et al. 2004; Harrison et al. 1996; Teshima et al. 2000; Tutel'ian et al. 1999; Zhu et al. 2004).

In glyphosate resistant soybean for example, the *cp4 epsps* bacterial transgene was introduced for resistance to the herbicide, glyphosate. To test its potential allergenicity, it was determined that the transgenic enzyme EPSPS is not homologous with any allergens; it is easily broken down, has no glycosylation sites in common with allergens and is not glycosylated in plants; makes up 0.02% of total protein; and is susceptible to heat. This analysis demonstrated the protein to be non-allergenic on the basis of ''weight of evidence'' (Padgette et al. 1996a; Duggan et al. 2000; Einspanier et al. 2001). Although in vitro tests with glyphosate resistant soybeans demonstrated that transgenic DNA had the potential to survive passage through the gut, the Quantitative Competitive PCR (QC-PCR) assay used in the study employed relatively short target sequences so it is likely that the DNA would be in such small fragments as to be of limited biological significance (Martin-Orue et al. 2002). Additionally, Sharma et al. (2006) demonstrated the uptake of low-copy endogenous and transgenic DNA fragments in gastrointestinal tract tissues and visceral tissue from transgenic canola (discussed earlier) and suggested that the likelihood of uptake of transgenic plant DNA into organ tissues of ruminant or monogastric animals is low.

Harrison et al. (1996) examined the acute toxicity of CP4 EPSPS protein by acute administration of CP4 EPSPS to mice by gavage at a high dosage of 572 mg/kg body weight, which exceeds the estimated consumption level for food products containing CP4 EPSPS protein by 1,000-fold. No adverse effects occurred in the mice dosed with CP4 EPSPS protein. Their body weight, cumulative body weight, and food consumption did not show significant differences between the control and CP4 EPSPS protein treated groups. Additionally, Lee et al. (2001) performed an in vitro test on mice to determine the toxicity of the CP4 EPSPS protein in maize. A high dose of 45.6 mg of transgenic EPSPS protein per kg body weight (BW) was administered orally each day for 90 days. There was no observed toxicity from the protein. Chang et al. (2003) subjected Sprague Dawley rats to a toxicity test by orally administering 0.5 or 2.0 mg per kg BW of CP4 EPSPS protein in saline solution three times per week for three weeks. The dosages of CP4 EPSPS protein in this study were considered to be the approximate amounts of EPSPS protein in soybean consumed annually by humans. No toxicity was observed when compared to the saline control. These results were expected since the CP4 EPSPS protein is readily digested in gastric and intestinal fluids in vitro and the protein is from a ubiquitous family of proteins with a history of safe consumption and no biological mechanism of toxicity to animals. The protein did not show meaningful amino acid sequence similarity when compared to known protein toxins (reviewed in Nair et al. 2002).

The assessment of allergenic potential using the aforementioned criteria was performed on the same Roundup Ready soybean event 40-3-2. The amino acid sequence of the CP4 EPSPS protein was found not to be homologous to any of the known allergens (Fuchs 1996). Additionally, this protein was shown to have no glycosylation sites in common with allergens and was not glycosylated in the plant (reviewed in Nair et al. 2002). The CP4 EPSPS protein was present at low levels, approximately 0.08% of the total protein, in whole Roundup Ready soybean seed (Padgette et al. 1994) and was susceptible to heat.

Passive cutaneous anaphylaxis reaction of the purified EPSPS protein was not observed in the Sprague Dawley rat system, whether administered orally or subcutaneously (Chang et al. 2003). Furthermore, addition of the EPSPS protein to cultures of sensitized peritoneal mast cells, or unsensitized but antisera-labeled mast cells, showed neither a remarkable change in histamine release nor any cytokine production, including interleukin-4 (IL-4) and tumor necrosis factor-α (TNF-α). Thus, Chang et al. (2003) concluded that the EPSPS protein in transgenic soybean did not exhibit any significant allergenicity. By using standard in vitro methods and a skin prick test for the determination of allergenicity, it was not possible to detect any significant difference in the allergenic potency between transgenic (CP4 EPSPS) and wild-type soybeans in soybean-sensitized patients (Sten et al. 2004).

In a lamb's gastric system, the half-life of CP4 EPSPS protein produced by glyphosate-tolerant canola was less than 15 s, and in the intestinal system it was less than 10 min. The human stomach is estimated to empty 50% of solid food in 2 h, and liquid empties in 25 min. This indicates that, if the CP4 EPSPS protein does not degrade in the human gastric system, it would most likely degrade in the intestinal system. The CP4 EPSPS protein was also inactivated by heating at 65°C for 15 min (Stanford

et al. 2003). These authors observed complete digestibility of the CP4 EPSPS protein in gastric fluid in 60 s. In vivo, in vitro, and ex vivo tests were performed on Sprague Dawley rats using glyphosate-tolerant soybean seeds. All three tests gave negative results, indicating that the allergenic potential of the CP4 EPSPS protein is very low. The CP4 EPSPS protein shows no homology with known allergens; therefore, glyphosate-tolerant crops containing the novel protein have low potential to cause allergies among humans and other animals. Native EPSPS proteins are normally present in foods and feeds derived from plant and microbial sources and the CP4 EPSPS protein is similar to these (Stanford et al. 2003).

Protein extracts prepared from transgenic maize (events MON810, Bt11, T25 and Bt176) and soybean (Roundup Ready) samples and from non-transgenic control samples were tested in skin prick in two sensitive groups: (i) children with food and inhalant allergy, and (ii) individuals with asthma-rhinitis. IgE immunoblot reactivity of sera from patients with food allergy to soybean (Roundup Ready) and maize (MON810, Bt11, Bt176) samples, as well as to the pure transgenic proteins (CryIA[b] and CP4 EPSPS) was evaluated. None of the individuals undergoing tests reacted differentially to the transgenic and non-transgenic samples under study. None of the volunteers tested exhibited detectable IgE antibodies against pure transgenic proteins (Batista et al. 2005).

Bar or phosphinothricin acetyl transferase (PAT)

Hérouet et al. (2005) concluded that no harm results from the inclusion of PAT proteins in human food or in animal feed, because PAT is highly specific and does not possess the characteristics associated with food toxins or allergens, i.e., it has no sequence homology with any known allergens or toxins; it has no N-glycosylation sites; it is rapidly degraded in gastric and intestinal fluids; and it is devoid of adverse effects in mice after intravenous administration at high doses. For PAT protein, a study was performed in which mice received 5,000 mg PAT/kg body weight (equivalent to 6,000 mg test material/kg). No effect on body weight and gross pathology was noted after two weeks. Bacterially produced recombinant PAT protein showed the same electrophoretic mobility as PAT expressed in maize event 1,507 developed for protection against lepidopteran pests. Levels of PAT protein were not quantifiable in kernels of 1,507 maize because they were below the limit of detection. For all these reasons event 1,507 was found to be safe (EFSA 2005). Similarly, the phosphinothricin acetyl transferase (PAT) protein conferring glufosinate tolerance to maize proved to be heat labile and highly and quickly degraded in simulated human and animal gastric fluid assays (Wehrman et al. 1996).

Wang et al. (2000) evaluated the safety of the *bar* gene in transgenic rice. They carried out acute toxicity experiments, mutation experiments and a 30-day feeding test. Rats consuming 16.32 and 64 g/kg BW grew/developed normally during the 30-day feeding trial. No abnormalities were detected in body weight, food utilization, blood components, ratio of organ to body weight, and pathohistological parameters at a dosage of $\leq$64 g/kg of transgenic rice consumed.

Conclusions

Man has been practising genetic modification of crops since the transition from hunter/gatherer to farmer. Conventional plant breeding involves the indiscriminate transfer of entire genomes from one plant to another without much control of the outcome. Admittedly, it is not often that conventional plant breeding breaks the species barrier, even though there are examples of this, e.g. Triticale, Tridordeum. Nonetheless, the effects of creating new cultivars through conventional methods, including ionizing radiation or chemical mutation, are largely unknown. It is not uncommon to employ cultivars with unknown characteristics in terms of toxins, and nutritional or allergenic components in conventional crop improvement programs. Yet despite this uncertainty, society views this type of genetic manipulation as acceptable and safe. There are many examples that demonstrate that products of conventional breeding can be unsafe and problematic, for example a conventionally produced potato variety that accumulated high levels of solanine in cool weather (Van Gelder et al. 1988) or an insect resistant celery

variety that was also produced using conventional technologies that accumulated high levels of psoralen inlight, causing skin burns (Ames and Gold 1999).

Transgenic plant releases and commercialization are governed by draconian rules unparalleled elsewhere in any other sector. The European Union in a report following a 15-year study (1985–2000) involving 400 public research institutions, at a cost of 70 million Euros stated ''... *genetically modified plants and products derived from them present no risk to human health or the environment......these crops and products are even safer than plants and products generated through conventional processes*'' (EC Research 2001). The claim that antibiotic resistance genes in transgenic plants will escape into natural bacterial populations that will subsequently become resistant to them, thus creating super-bacteria is at best odd, as these genes are already present in the bacterial population in nature. It is worth remembering that the selectable marker genes were isolated from these very naturally occurring bacteria in the first place, for use in the laboratory.

Technology developers must make choices about product design, including transformation technologies and selectable marker systems, in order to maximize worldwide acceptability of the resulting products. Sometimes that means foregoing the use of antibiotic resistance marker genes for technologies with fewer burdens. Unless and until industrial technology developers agree to fight for acceptance of antibiotic resistance selectable marker systems, this fight belongs solely to academics, who have the luxury of arguing for their use based on safety studies alone (Scott Thenell, personal communication).

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References

Ames BN, Gold LS (1999). In: Morris, Bate R (eds) In Fearing Food. Oxford, Butterworth Heinemann, pp 18–38

Ash JA, Scheideler E, Novak CL (2000) The fate of genetically modified protein from Roundup Ready® soybeans in the laying hen. *Poultry Sciences* 1:26; Palais de Congrès, Montreal Canada; August 18–21, 2000

Ash J, Novak C, Scheideler SE (2003) The fate of genetically modified protein from Roundup Ready soybeans in the laying hens. J Appl Poult Res 12:242–245

Aulrich K, Böehme H, Daenicke R, Halle I, Flachowsky G (2002) Novel feeds—a review of experiments at our institute. Food Res Int 35:285–293

Aumaitre A (2004) Safety assessment and feeding value for pigs, poultry and ruminant animals of pest protected (Bt) plants and herbicide tolerant (glyphosate, glufosinate) plants: interpretation of experimental results observed worldwide on GM plants. Ital J Anim Sci 3: 107–121

Aumaitre A, Aulrich K, Chesson A, Flachowsky G, Piva G (2002) New feeds from genetically modified plants: substantial equivalence, nutritional equivalence, digestibility, and safety for animals and the food chain. Livest Prod Sci 74:223–238

Baker JM, Hawkins ND, Ward JL, Lovegrove A, Napier JA, Shewry P, Beale MH (2006) A metabolomic study of substantial equivalence of field-grown genetically modified wheat. Plant Biotechnol J 4:381–392

Batista R, Nunes B, Carmo M, Cardoso C, José H, de Almeida A, Manique A, Bento L, Ricardo C, Oliveira M (2005) Lack of detectable allergenicity of transgenic maize and soya samples. J Allergy Clin Immunol 116:403–410

Baudo MM, Lyons R, Powers S, Pastori GM, Edwards KJ, Holdsworth MJ, Shewry PR (2006) Transgenesis has less impact on the transcriptome of wheat grain than conventional breeding. Plant Biotechnol J 4:369–380

Bennett PM, Livesey CT, Nathwani D, Reeves DS, Saunders JR, Wise R (2004) An assessment of the risks associated with the use of antibiotic resistance genes in genetically modified plants: report of the Workig Party of the British Society for Antimicrobial Chemotherapy. J Antimicrob Chemother 53:418–431

Bertolla F, Simonet P (1999) Horizontal gene transfers in the environment: natural transformation as a putative process for gene transfers between trangenic plants and microorganisms. Res Microbiol 150(6):375–384

Bertrand JA, Sudduth TQ, Condon A, Jenkins TC, Calhoun MC (2005) Nutrient content of whole cottonseed. J Dairy Sci 88:1470–1477

Blaese RM, Anderson WF, Culver KW (1990) Treatment of severe combined immunodeficiency disease (SCID) due to adenosine deaminase (ADA) deficiency with autologous lymphocytes transduced with a human ADA gene. Hum Gene Ther 1:327

Bohme H, Aulrich K, Daenicke R, Flachowsky G (2001) Genetically modified feeds in animal nutrition. 2nd communication: glufosinate tolerant sugarbeet (roots and silage) and maize grains for ruminants and pigs. Arch Tierernahr 54(3):197–207

Brake J, Faust MA, Stein J (2003) Evaluation of transgenic event Bt11 hybrid corn in broiler chickens. Poultry Sci 82(4):551–559

Brake DG, Evenson DP (2004) A generational study of glyphosate-tolerant soybeans on mouse fetal, postnatal, pubertal and adult testicular development. Food Chem Toxicol 42:29–36

Brookes F, Barfoot P (2005) GM Crops: the global economic and environmental impact–the first nine years 1996–2004. AgBioForum 8:187–196

Burks A, Fuchs R (1995) Assessment of the endogenous allergens in glyphosate-tolerant and commercial soybean varieties. J Allergy Clin Immunol 96(6): 1008–1010

Council for Agricultural Science and Technology (CAST). 2006. Safety of meat, milk, and eggs from animals fed crops derived from modern biotechnology. Issue paper 34. CAST, Ames, Iowa

Castillo AR, Gallardo MR, Maciel M, Giordano JM, Conti GA, Gaggiotti MC, Quaino O, Gianni C, Hartnell GF (2004) Effects of feeding rations with genetically modified whole cottonseed to lactating dairy cows. J Dairy Sci 87: 1778–1785

Chambers PA, Duggan PS, Heritage J, Forbers JM (2002) The fate of antibiotic resistance marker genes in transgenic plant feed material fed to chickens. J Antimicrob Chemother 49:161–164

Chang HS, Kim NH, Park MJ, Lim SK, Kim SC, Kim JY, Kim JA, Oh HY, Lee CH, Huh K, Jeong TC, Nam DH (2003) The 5-enolpyruvylshikimate-3-phosphate synthase of glyphosate-tolerant soybean expressed in *Escherichia coli* shows no severe allergenicity. Mol Cells 15(1): 20–6

Chesson A, Flachowsky G (2003) Transgenic plants in poultry nutrition. Worlds Poult Sci J 59(2): 201–207

Christou P, Ford TL, Kofron M (1991) Genotype-independent stable transformation of rice (*Oryza sativa*) plants. Biotechnology (N Y) 9:957–962

Christou P (1996) Transformation technology. Trends Plant Sci 1:423–431

Christou P, Twyman RM (2004) The potential of genetically enhanced plants to address food insecurity. Nutr Res Rev 17:23–42

Christou P, Capell T, Kohli A, Gatehouse JA, Gatehouse AMR (2006) Recent developments and future prospects in insect pest control in transgenic crops. Trends Plant Sci 11: 302–308

Clark JH, Ipharraguerre IR (2001) Livestock performance: Feeding biotech crops. J Dairy Sci 84(E. Suppl.):E9–E18

Codex Alimentarius Commission (Codex) (2001) Codex guidelines (ALINORM 01/22). FAO/WHO, Rome, Italy. Available on the World Wide Web: http://www.codexalimentarius.net/

Cromwell GL, Lindemann MD, Randolph JH, Parker GR, Coffey RD, Laurent KM, Armstrong CL, Mikel WB, Stanisiewski EP, Hartnell GF (2002) Soybean meal from Roundup Ready or convencional soybeans in diets for growing-finishing swine. J Anim Sci 80:708–715

Cromwell GL, Henry BJ, Scout AL, Gerngross MF, Dusek DL, Fletcher DW (2005) Glufosinate herbicide-tolerant (LiberyLink) rice vs. Convencional rice in diets for growing-finishing swine. J Anim Sci 83:1068–1074

Dale PJ, Clarke B, Fontes EMG (2002) Potential for the environmental impact of transgenic crops. Nat Biotechol 20:567–574

DANMAP (2001) Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, foods and humans in Denmark. ISSN 1600–2032

D'Costa VM, McGrann KM, Hughes DW, Wright GD (2006) Sampling the antibiotic resistome. Science 311:374–377

de Vries J, Heine M, Harms K, Wackernagel W (2003) Spread of recombinant DNA by roots and pollen of transgenic potato plants, identified by highly specific biomonitoring using natural transformation of an *Acinetobacter* sp. Appl Environ Microbiol 69:4455–4462

Donkin SS, Velez JC, Totten AK, Stanisiewski EP, Hartnell GF (2003) Effects of feeding silage and grain from glyphosate-tolerant or insect-protected corn hybrids on feed intake ruminal digestión, and milk production in dairy cattle. J Dairy Sci 86:1780–1788

Duggan PS, Chambers PA, Heritage J amd Forbes JM (2000) Survival of free DNA encoding antibiotic resistance from transgenic maize and the transformation activity of DNA in ovine saliva, ovine rumen fluid and silage effluent. FEMS Microbiol Lett 191:71–77

EC (2001) Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC. Official J Eur Commun L106:1–39. http://europa.eu.int/eur-lex/en/lif/dat/2001/en_301L0018.html

EC (2002) Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. Official Journal of the European Communities L31, 1–24. http://europa.eu.int/eur-lex/pri/en/oj/dat/2002/l_031/l_03120020201en00010024.pdf

EC (2003) European Commission, Health & Consumer protection directorate-general: guidance document for the risk assessment of genetically modified plants and derived food and feed. 6–7 March 2003

EC Research (2001) EC-sponsored research on safety of genetically modified organisms: a review of results, http://europa.eu.int/comm/research/quality-of-life/gmo/

EFB (2001) European Federation of Biotechnology. Antibiotic resistance markers in genetically modified (GM) crops, task group on public perceptions of biotechnology briefing paper 10, September 2001

EFSA (2004a) Guidance document of the scientific panel on genetically modified organisms for the risk assessment of genetically modified plants and derived food and feed. The EFSA Journal 99:1–94

EFSA (2004b) Opinion of the scientific panel on genetically modified organisms on the use of antibiotic resistance genes as marker genes in genetically modified plants. EFSA J 48:1–18

EFSA (2005) Opinion of the scientific panel on genetically modified organisms on a request from the Commission related to the notification (Reference C/ES/01/01) for the placing on the market of insect-tolerant genetically modified maize 1507, for import, feed and industrial processing and cultivation, under Part C of Directive 2001/18/EC from Pioneer Hi-Bred International/Mycogen Seeds. The EFSA Journal 181: 1–33

Einspanier R, Klotz A, Kraft J, Aulrich K, Poser R, Schwagele F, Jahreis G, Flachowski G (2001) The fate of forage plant DNA in farm animals: a collaborative case-study investigating cattle and chicken fed recombinant plant material. Eur Food Res Technol 212:129–134

Entransfood, the European network on safety assessment of genetically modified food crops (2004) Genetically modified crops in the EU: food safety assessment, regulation, and public concerns. Overarching report. European commission. (Site accessed May 2006) http://www.entransfood.com/

EU Regulation 1829/2003: EU rules for the authorization and monitoring of genetically modified food and feed (ex 258/97); EU Regulation 1830/2003: traceability and labelling of genetically modified organisms (ex 49 and 50/2000). http://europa.eu.int/eurlex

FAO (2001) Statistics on food production and consumption, FAO Food balance sheets. http://faostat.fao.org/

FAO/WHO (1996) Biotechnology and food safety. Report of a joint Food and Agriculture Organisation/World Health Organisation Consultation. FAO/WHO: Rome

FAO/WHO (2000) Safety aspects of genetically modified foods of plant origin, FAO/WHO consultation 29 May–2 June 2000. World Health Organization, Geneva, Switzerland

FASS (2006) Federation of animal science societies. (Site accessed May 2006) http://www.fass.org/

Faust M, Miller L (1997) Study finds no Bt in milk. IC-478. Fall special livestock edition. Iowa State University Extension, Ames, Iowa, pp 6–7

FDA (1992) Statement of policy: foods derived from new plant varieties. U.S. Food and Drug Administration. US Fed Regist 57(104):22984–23005

FDA (1998) Guidance for industry: use of antibiotic resistance marker genes in transgenic plants. U.S. Food and Drug Administration. Downloaded from http://vm.cfsan.fda.gov/

FDA/CFSAN (1998) Draft guidance for industry: use of antibiotic resistance marker genes in transgenic plants—U. S. FDA Center for Food Safety and Applied Nutrition (Office of Premarket Approval), September 4, 1998

Ferry N, Edwards MG, Gatehouse J, Capell T, Christou P, Gatehouse AMR (2006) Transgenic plants for insect pest control. A forward looking scientific perspective Transgenic Res 15:13–19

Fink R, Moran E (2005) Biosafety for large-scale containment level 1 operations using recombinant DNA technology: no emerging hazards. Appl Biosafety 10(1):30–39

Flachowsky G, Aulrich K (2001) Nutritional assessment of feeds from genetically modified organism. J Anim Feed Sci 10(1):181–194

Flachowsky G, Aulrich K (2002) Food of animal origin after feeding of feeds from genetically modified plants (GMP). Ernahrungs-Umschau 49(3):84–88

Flavell R, Dart E, Fuchs R, Fraley F (1992) Selectable marker genes: safe for plants? Biol Technol 10:141–142

Food Additive Petition (1993) Aminoglycoside 3′-Phosphotransferase II (APH(3′)II): safety and use in the production of genetically engineered plants, Calgene, Inc., Volume II: Environmental Assessment, Number 3A4364, June 3, 1993

Frank SA (1994) Polymorphism of bacterial restriction modification systems: the advantage of diversity. Evolution 48:1470

FSANZ, Food Standards Australia New Zealand (2003) Oil derived from glufosinate-ammonium tolerant and pollination controlled canola: a safety assessment. Technical report: series no. 16

Fuchs RL (1996) In: Eisenbrand G, Aulepp H, Dayan AD, Elias PS, Grunow W, Ring J, Schlatter J, Köhl W, Baum M (eds) Assessment of the allergenic potential of foods derived from genetically engineered plants: glyphosate tolerant soybean as a case study, ch. 17. Food allergies and intolerances symposium. VCH Verlagsgesellschaft mbH, Weinheim, Germany, pp 212–221

Fuchs RL, Ream JE, Hammond BG, Naylor MW, Leimgruber RM, Berberich SA (1993) Safety assessment of the neomycin phosphotransferase-II (NPTII) protein. Bio/Technol 11:1543–1547

Garfinkel DJ, Simpson RB, Ream LW, White FF, Gordon MP, Nester EW (1981) Genetic analysis of crown gall; fine structure map of the T-DNA by site-directed mutagenesis. Cell 27:143–153

Gay P, Gillespie S (2005) Antibiotic resistance markers in genetically modified plants: a risk to human health. Lancet Infect Dis 5:637–646

Gebhard F, Smalla K (1998) Transformation of *Acinetobacter* sp. strain BD413 by transgenic sugar beet DNA. Appl Environ Microbiol 64:1550–1554

Gebhard F, Smalla K (1999) Monitoring field releases of genetically modified sugar beets for persistence of transgenic plant DNA and horizontal gene transfer. FEMS Microbiol Ecol 28:261–272

Graham RD, Welch RM, Bouis HE (2001) Addressing micronutrient malnutrition through enhancing the nutritional quality of staple foods: principles, perspectives and knowledge gaps. Adv Agro 70:77–142

Grant RJ, Fanning KC, Kleinschmit D, Stanisiewski EP, Hartnell GF (2003) Influence of glyphosate-tolerant (event nk 603) and corn rootworm protected (event MON863)corn silage and grain on feed consumption and milk production in holstein cattle. J Dairy Sci 86: 1707–1715

Gritz L, Davis J (1983) Plasmid-encoded hygromycin B resistance: the sequence of hygromycin B phosphotransferase gene and its expression in *Escherichia coli* and *Saccharomyces cerevisiae*. Gene 25:179–188

Halford NG, Shewry PR (2000) Genetically modified crops: methodology, benefits, regulation and public concerns. Br Med Bull 56(1):62–73

Hamilton KA, Pyla PD, Breeze M, Olson T, Li M, Robinson E, Gallagher SP, Sorbet R, Chen Y (2004) Bollgard II cotton: compositional analysis and feeding studies of cottonseed from insect-protected cotton (*Gossypium hirsutum* L.) producing the Cry1Ac and Cry2Ab2 proteins. J Agric Food Chem 52:6969–6976

Hammond BG, Vicini JL, Hartnell GF, Naylor MW, Knight CD, Robinson E, Fuchs RL, Padgette SR (1996) The feeding value of soybeans fed to rats, chickens, catfish and dairy cattle is not altered by incorporation of glyphosate tolerance. J Nutr 126:717–727

Hammond B, Dudek R, Lemen J, Nemeth M (2004) Results of a 13 week safety assurance study with rats fed grain from glyphosate tolerant corn. Food Chem Toxicol 42: 1003–1014

Harrison LA, Bailey MR, Naylor MW, Ream JE, Hammond BG, Nida DL, Burnette BL, Nickson TE, Mitsky TA,

Taylor ML, Fuchs RL, Padgette SR (1996) The expressed protein in glyphosate-tolerant soybean, 5-enolpyruvylshikimate-3-phosphate synthase from Agrobacterium sp. strain CP4, is rapidly digested in vitro and is not toxic to acutely gavaged mice. J Nutriti 126:728–740

Hartnell GF, Hvelplund T, Weisbjerg MR (2005) Nutrient digestibility in sheep fed diets containing Roundup Ready or conventional fodder beet, sugar beet, and beet pulp. J Anim Sci 83:400–407

Hérouet C, Esdaile DJ, Mallyon BA, Debruyne E, Schulz A, Currier T, Hendrickx K, van der Klis R, Rouan D (2005) Safety evaluation of the phosphinothricin acetyltransferase proteins encoded by the pat and bar sequences that confer tolerance to glufosinate-ammonium herbicide in transgenic plants. Reg Toxic Pharm 41:134–149

High SM, Cohen MB, Shu QY, Altosaar I (2004) Achieving successful deployment of Bt rice. Trends Plant Sci 9(6):286–292

Huang J, Pray C, Rozelle S (2002) Enhancing the crops that feed the poor. Nature 418:618–684

Huang J, Hu R, Rozelle S, Pray C (2005) Insect resistant GM rice in farmers fields: assessing productivity and health effects in China. Science 308:688–690

Hyun Y, Bressner GE, Fischer RL, Miller PS, Ellis M, Peterson BA, Stanisiewski EP, Hartnell GF (2005) Performance of growing-finishing pigs fed diets containing YieldGard Rootworm corn (MON 863), a non transgenic genetically similar corn, or conventional corn hybrids. J Anim Sci 83:1581–1590

Ipharraguerre IR, Younker RS, Clark JH, Stanisiewski EP, Hartnell GF (2003) Performance of lactating dairy cows fed corn as whole plant silage and grain produced form a glyphosate-tolerant hybrid (event NK603). J Dairy Sci 86:1734–1741

Jacoby GA, Medeiros AA (1991) More extended-spectrum β-lactamases. Antimicrob Agents Chemother 35:1697–1704

James C (2005) Preview: Global Status of Commercialized Biotech/GM Crops ISAAA Briefs No 34. ISAAA: Ithaca, NY http://www.africabio.com/pdf/Briefs%2034.pdf

Jennings JC, Kolwyck DC, Kays SB, Whetsell AJ, Surber JB, Cromwell GL, Lirette RP, Glenn KC (2003) Determining whether transgenic and endogenous plant DNA and transgenic protein are detectable in muscle from swine fed roundup ready soybean meal. J Anim Sci 81: 1447–1455

Jonas DA, Elmadfa I, Engel KH, Heller KJ, Kozianowski G, Konig A, Muller D, Narbonne JF, Wackernagel W, Kleiner J (2001) Safety considerations of DNA in food. Ann Nutr Metab 45:235–254

Kan C, Hartnell G (2004) Evaluation of broiler performance when fed roundup-ready wheat (event MON 71800), control, and commercial wheat varieties. Poultry Sci 83: 1325–1333

Kasid A, Morecki S, Aebersold P, Kasid A, Morecki S, Aebersold P, Cornetta K, Culver K, Freeman S, Director E, Lotze MT, Blaese RM, Anderson WF (1990) Human gene transfer: Characterization of human tumor-infiltrating lymphocytes as vehicles for retroviral-mediated gene transfer in man. Proc Natl Acad Sci 87:473–477

Kay E, Vogel TM, Bertola F, Nalin R, Simonet P (2002) In situ transfer of antibiotic resistance genes from transgenic (transplastomic) tobacco plants to bacteria. Appl Env Microbiol 68:3345–3351

Kok EJ, Kuiper HA (2003) Comparative safety assessment for biotech crops. *Trends in Biotechn*ol 21:439–444

Kuhstoss S, Rao RN (1983) Expression in *Streptomyces ambofaciens* of an *Escherichia coli* K-12 gene which confers resistance to hygromycin B. Gene 26:295–299

Leboul J, Davies J (1982) Enzymatic modification of hygromycin B in *Streptomyces hygroscopicus*. J Antibiot 35:527–528

Lee T, Leach J, Ledesma B, Hammond B, Naylor M, Fuchs R, Sindhu R, Rodriguez D, Aswood J (2001) Safety assessment of MEPSPS protein in roundup ready corn plants. Proc Soc Toxicol Annu Meet: 411

MacKenzie D (1999) Unpalatable truths. New Scientist 162:18–19

MacKenzie D, McLean M (2002) Who's afraid of GM feeds? Feed Mix 10(3): 16–19

Malpartida F, Zalacain M, Jimenen A, Davies J (1983) Molecular cloning and expression in *Streptomyces lividans* of a hygromycin B phosphotransferase geen from *Streptomyces hygroscopicus*. Biochem Biophys Res Commun 117:6–12

Martin-Orue SM, O'Donnell AG, Arin∼o J, Netherwood T, Gilbert HJ, Mathers JC (2002) Degradation of transgenic DNA from genetically modified soya and maize in human intestinal simulations. Bri J Nutr 87(6):533–542

Maryanski JH (1995) Food and Drug Administration policy for foods developed by biotechnology. In: Engel K-H, Takeoka GR, Teranishi R (eds) Genetically modified foods: safety issues. ACS Symposium Series No. 605:12–22. American Chemical Society, Washington, DC

McAllan AB (1980) The degradation of nucleic acids in, and the removal of breakdown products form the small intestine of steers. Br J Nutr 44:99–112

McAllan AB (1982) The fate of nucleic acids in ruminants. Proc Nutr Soc 41(3):309–317

McCabe D.E., Swain W.F., Martinell B.J., Christou P (1988) Stable transformation of soybean (Glycine max) by particle acceleration. Bio Tech 6:923–926

Miki B, McHugh S (2004) Selectable marker genes in transgenic plants: applications, alternatives and biosafety. J Biotech 107:193–232

MRC (2000) Report of a Medical Research Council expert group on genetically modified (GM) foods. Medical Research Council: London

Munkvold GP, RL Hellmich WB Showers (1997) Reduced Fusarium ear rot and symptomless infection in kernels of maize genetically engineered for European corn borer resistance. Phytopathol 87:1071–1077

Munkvold GP, Hellmich RL, Rice LG (1999) Comparison of fumonisin concentration in kernels of transgenic Bt maize hybrids and non-transgenic hybrids. Plant Dis 83:130–138

Murray IA, Shaw WV (1997) *O*-cetyltransferases for chloramphenicol and other natural products. Antimicrob Agents Chemother 41:1–6

Nair RS, Fuchs RL, Schuette SA (2002), Genetically modified food: hazard identification and risk assessment: current methods for assessing safety of genetically modified crops as exemplified by data on roundup ready1 soybeans. Toxicol Pathol 30(1):117–125

Nap JP, Bijvoet J, Stikema WJ (1992) Biosafety of kanamycin-resistant transgenic plants: an overview. Transg Res 1:239–249

Nida DL, Patzer S, Harvey P, Stipanovic R, Wood R, Fuchs RL (1996) Glyphosate-tolerant cotton: The composition of the cottonseed is equivalent to that of conventional cottonseed. J Agri Food Chem 44(7):1967–1974

Nielsen KM, Bones AM, Smalla K, van Elsas JD (1998) Horizontal gene transfer from transgenic plants to terrestrial bacteria—a rare event? FEMS Microbiol Rev 22: 79–103

OECD (1993), Safety evaluation of foods produced by modern biotechnology—concepts and principles. Organization for Economic Cooperation and Development, Paris

OECD (2000) GM food safety: facts, uncertainties and assessment. In: OECD Edinburgh conference on the scientific and health aspects of genetically modified foods, Edinburgh. OECD, Paris

Padgette SR, Re DB, Hammond BG, Fuchs RL, Rogers SG, Harrison LA, Nida DL, Naylor MW, Kolacz KH, Taylor NB and Ream JE (1994) Safety, compositional and nutritional aspects of glyphosate-tolerant soybeans: conclusion based on studies and information evaluated according to FDA's consultation process. Monsanto Company, St. Louis: 1–107

Padgette SR, Taylor NB, Nida DL, Bailey MR, MacDonald J, Holden LR, Fuchs RL (1996a) The composition of glyphosate-tolerant soybean seeds is equivalent to that of conventional soybeans. J Nutriti 126(3):702–716

Padgette, SR, Re DB, Barry GF, Eichholtz, DE, Delannay X, Fuchs RL, Kishore GM, Fraley RT (1996b) New weed control opportunities: development of soybeans with a Roundup Ready® gene. In: Duke SO (ed) Herbicide resistant crops. CRC Press, Boca Raton, FL, pp 53–84

Pastorello EA, Conti A, Pravettoni V, Farioli L, Rivolta F, Ansaloni R, Ispano M, Incorvaia C, Giuffrida MG, Ortolani C (1998) Identification of actinidin as the major allergen of kiwi fruit. J Allergy Clin Immunol 101:531–537

Phipps RH, Beever DE, Humphries DJ (2002) Detection of transgenic DNA in milk from cows receiving herbicide tolerant (CP4 EPSPS) soyabean meal. Livest Prod Sci 74:269–273

Phipps RH, Deaville ER, Maddison BC (2003) Detection of transgenic and endogenous plant DNA in rumen fluid, duodenal digesta, milk, blood, and feces of lactating dairy cows. J Dairy Sci 86:4070–4078

Phipps RH, Jones AK, Tingey AP, Abeyasekera S (2005) Effect of corn silage from an herbicide-tolerant genetically modified variety on milk production and absence of transgenic DNA in milk. J Dairy Sci 88:2870–2878

Pinstrup-Andersen P, Pandya-Lorch R, Rosegrant MW (1999) World food prospects: critical issues for the twenty-first century. Washington, IFPRI

Proctor GN, Rownd RH (1982) Rosanilins. Indicator dyes for chloramphenicol-resistant enterobacteria containing chloramphenicol acetyltransferase. J Bacteriol 150: 1375–1382

Rao RN, Allen NE, Hobbs JN, Alborn WE, Kirst HA, Paschal JW (1983) Genetic and enzymatic basis of hygromycin B resistance in *Escherichia coli*. Antimicrob Agents Chemother 24:689–695

Redenbaugh K, Hiatt W, Martineau B, Lindemann J, Emlay D (1994) Aminoglycoside 3′-phosphotransferase II (APH(3′)II): Review of its safety and use in the production of genetically engineered plants. Food Biotechnol 8:137–165

Royal Society (2002) Genetically modified plants for food use and human health-an update. Policy document 4/02. Royal Society: London

Royal Society of Canada (2001) Elements of precaution: recommendations for the regulation of food biotechnology in Canada (Report of the expert panel on the future of food biotechnology). Royal Society of Canada, Ottawa

Sanden M, Krogdahl A, Bakke-Mckellep AM, Buddington RK (2006) Growth performance and organ development in Atlantic salmon, *Salmo salar* L. parr fed genetically modified (GM) soybean and maize. Aquac Nutr 12:1–14

Sanhoty RE, Abd El-Rahman AA, Bögl KW (2004) Quality and safety evaluation of genetically modified potatoes Spunta with Cry V gene: Compositional analysis, determination of some toxins, antinutrients compounds and feeding study in rats. Nahrung/Food 48(1): 13–18

Sharma R, Alexander TW, John SJ, Forster RJ, McAllister TA (2004) Relative stability of transgene DNA fragments from GM rapeseed in mixed ruminal cultures. Br J Nutr 91:673–681

Sharma R, Damgaard D, Alexander TW, Dugan MER, Aalhus JL, Stanford K, McAllister TA (2006) Detection of transgenic and endogenous plant DNA in digesta and tissues of sheep and pigs fed roundup ready canola meal. J Agric Food Chem 54:1699–1709

Shaw KJ, Rather PN, Hare RS, Miller GH (1993) Molecular genetics of aminoglycoside resistance genes and familiar relationships of the aminoglycoside-modifying enzymes. Microbiol Rev 57:138–163

Sidhu RS, Hammond BG, Fuchs RL, Mutz JN, Holden LR, George B, Olson T (2000) Glyphosate-tolerant corn: The composition and feeding value of grain from glyphosate-tolerant corn is equivalent to that of conventional corn (*Zea mays L.*). J Agric Food Chem 48:2305–2312

Smalla K, Borin S, Heuer H, Gebhard F, van Elsas JD and Neilson K (2000) Horizontal transfer of antibiotic resistance genes from transgenic plants to bacteria. Are there new data to fuel the debate? In: Fairbairn G, Scoles G, McHugen A (eds) Proceeding of the 6th international symposium on the biosafety of genetically modified organisms. pp 146–154

Standford K, Aalhus JL, Dugan MER, Wallins GL, Sharma R, McAllister TA (2003) Effects of feeding transgenic canola on apparent digestibility, growth performance and carcass characteristics of lambs. Can J Anim Sci 83:299–305

Sten E, Skov PS, Andersen SB, Torp AM, Olesen A, Bindslev-Jensen U, Poulsen LK, Bindslev-Jensen C (2004) A comparative study of the allergenic potency of wild-type and glyphosate-tolerant gene-modified soybean cultivars. APMIS 112:21–28

Stoger E, Sack M, Perrin Y, Vaquero C, Torres E, Twyman R, Christou P, Fischer R (2002) Practical considerations for pharmaceutical antibody production in different crop systems. Mol Breed 9:149–158

Syvanen M (1999) In search of horizontal gene transfer. Nat Biotechnol 17:833

Taylor ML, Hyun Y, Hartnell GF, Riordan SG, Nemeth MA, Karunanandaa K, George B, Astwood JD (2003) Comparison of broiler performance when fed diets containing grain from YieldGard Rootworm (MON 863), YieldGard Plus (MON 810xMON863), nontransgenic control, or commercial reference corn hybrids. Poult Sci 82:1948–1956

Taylor M, Stanisiewski E, Riordan S, Nemeth M, George B, Hartnell G (2004) Comparison of broiler performance when feds diets containing Roundup Ready (Event RT73), nontransgenic control, or commercial canola meal. Poult Sci 83:456–461

Taylor ML, Hartnell G, Nemeth M, Karunanandaa K, George B (2005) Comparison of broiler performance when fed diets containing corn grain with insect-protected (corn rootworm and European corn borer) and herbicide-tolerant (glyphosate) traits, control corn, or commercial reference corn. Poult Sci 84:587–593

Tepfer D, Garcia-Gonzales R, Mansouri H, Seruga M, Message B, Leach F, Perica MC (2003) Homology-dependent DNA transfer from plants to a soil bacterium under aboratory conditions: implications in evolution and horizontal gene transfer. Transgenic Res 12:425–437

Teshima R, Akiyama H, Okunuki H, Sakushima JI, Goda Y, Onodera H, Sawada JI, Toyoda M (2000) Effect of GM and non-GM soybeans on the immune system of BN rats and B10A mice. J Food Hyg Soc Jpn 41:188–193

Toenniessen GH, Ó Tool JC, DeVries J (2003) Advances in Plant Biotechnology and its Adoption in Developing Countries. Curr Opin Plant Biol 6:191–198

Tomalsky ME, Crosa JH (1987) Tn1331, a novel multiresistant transposon encoding resistance to amikasin and ampicillin in *Klebsiella pneumoniae*. Antimicrob Agents Chemother 31:1955–1960

Tutel'ian VA, Kravchenko LV, Lashneva NV, Avren'eva Ll, Guseva GV, Sorokina EIu, Chernysheva ON (1999) Medical and biological evaluation of safety of protein concentrate from genetically-modified soybeans. Biochemical studies. Vopr Pitan 68:9–12

UNDP (2001) United Nations Development Programme, Human Development Report 2001: Making new technologies work for human development. Oxford University Press, New York, pp 65–78

Van Gelder WMJ, Vinke JH, Scheffer JJC (1988) Steroidal glycoalkaloids in tubers and leaves of solanum species used in potato breeding. Euphytica 38:147–158

Wang Y, Lai W, Chen J, Mei S (2000) Toxicity of anti-herbicide gene (BAR) transgenic rice. Wei Sheng Yan Jiu 29(3): 141–142

Weber TE, Richert BT, Kendall DC, Bowers KA and Herr CT (2000) Grower-finisher performance and carcass characteristics of pigs fed genetically modified ''Bt'' corn. J Anim Sci 79(2):67(Abstr 162). Purdue university. Swine day

Wehrman A, Van Vliet A, Opsomer C, Botternan J, Schulz A (1996) The similarities of *bar* and *pat* gene products make them equally applicable for plant engineers. Nat Biotechnol 14:1274–1278

Wu F (2006) Mycotoxin reduction in Bt corn: potential economic, health, and regulatory impacts. Transgen Res 15:277–289

Zhu YZ, Li DF, Wang FL, Yin JD, Jin H (2004) Nutritional assessment and fate of DNA of soybean meal from roundup ready or conventional soybeans using rats. Arch Anim Nutr 58:295–310