

Sources and Mechanisms of Health Risks from Genetically Modified Crops and Foods

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Genetic modification (GM) is a purely laboratory-based method that exploits the use of recombinant DNA or genetic engineering technology to produce novel varieties of crops. It represents a radically different approach to new crop production when compared to traditional plant breeding methods, and even those using approaches such as irradiation and chemical-induced mutation. The artificial nature of GM does not automatically make it dangerous and undesirable. It is the outcome of the GM process that gives cause for concern. GM allows the transfer of any gene from any source into a crop, thereby bringing about combinations of genes that would not occur naturally. In addition, the GM transformation process as a whole is highly mutagenic. These generic properties of GM combine to generate a high risk of disturbing plant host gene function and biochemistry that could result in

novel toxin and allergen production as well as a compromised nutritional value (for review see Antoniou et al., 2012).

There are three sources of health risks that can potentially arise from GM foods:

1. The introduced foreign GM gene ('transgene'):
 - (a) GM gene product directly (e.g. Bt toxin);
 - (b) Altered plant biochemistry caused by GM gene product (e.g. enzymes conferring herbicide tolerance);
2. Higher exposures to herbicides used in conjunction with the cultivation of GM crops (e.g. glyphosate);
3. Altered plant biochemistry caused by mutagenic effect of the GM transformation process.

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This paper will focus primarily on illustrating potential sources of harm arising from points 1(a) and 3 above.

Feeding studies for evaluating toxicity of GM crops

There are just four major GM crops grown commercially in the world today, three of which are feed and food crops. These are soybeans, maize or corn, canola and cotton, which collectively constitute approximately 10% of global agriculture with cultivation concentrated in North and South America. All the GM soy is engineered to be tolerant to glyphosate-based herbicide (mostly Roundup formulations) applications. Of these four GM crops, two are predominantly engineered to express versions of the insecticidal Bt toxin protein. These are corn and cotton. It should be noted that some varieties of GM corn and cotton are engineered to express both a Bt toxin and be tolerant to glyphosate. Feeding trials in established laboratory animal model systems (rats, mice) which have been routinely used to evaluate potential human toxicity have been conducted with various varieties of commercialised and non-commercialised GM crops. Although not all published animal feeding studies of this type have shown disturbances to physiological and biochemical function with potential negative health outcomes (e.g. Liu et al., 2012), many have shown very worrying results. The findings of the studies from both industry and academia that give rise to cause for concern are summarised below.

Studies conducted by industry

Although not mandatory, some regulators (especially within the European Union) request feeding studies with rats to evaluate potential toxicity of a GM crop as part of the industry's application for marketing approval. These studies are based on Organisation for Economic Co-operation and Development (OECD) guidelines and thus are of only 90 days' duration. Nevertheless, independent academic re-evaluation of the results from these short-term feeding trials has shown:

- Rats fed insecticide-producing MON863 Bt corn grew more slowly and showed higher levels of certain fats (triglycerides) in their blood than rats fed the control diet. They also suffered problems with liver and kidney function. The authors stated that it could not be concluded that MON863 corn is safe and that long-term studies were needed to investigate the consequences of these effects (Séralini et al., 2007).
- Rats fed commercialised GM Bt corn varieties MON863 and MON810 as well as Roundup-tolerant NK603, had toxic effects on liver and kidneys. The authors of the re-analysis stated that while the findings may have been due to the pesticides specific to each variety, genetic engineering could not be excluded as the cause (de Vendomois et al., 2009).
- Various animals were fed Bt toxin-containing brinjal ('Bt brinjal') for a maximum of 90 days (rats, rabbits, goats) or 42-45 days (cows, chickens, fish). Despite the short duration of these feeding tests the results showed significant signs of toxicity to multiple organ systems in the Bt brinjal groups compared to the non-GM brinjal controls; e.g., less feed consumption in goats and rabbits; diarrhoea, higher water consumption, liver and body weight decrease in rats; clear signs of disruption in liver function in rabbits and goats; disturbances in pancreatic, kidney and haematological function in rabbits.

Taken together, the data from these industry studies show statistically significant differences in the function of multiple organ systems between the GM and equivalent non-GM control feeding groups. There are evidently clear signs of toxicity especially with respect to liver and kidney function. Although not providing clear evidence of harm, they also do not provide clear evidence of safety.

Although these statistically significant findings with GM corn were subsequently acknowledged by both industry and EU regulators, they were dismissed as 'biologically insignificant', a scientifically meaningless term without definition. Therefore, rather

than commissioning longer, life-long feeding trials to ascertain whether the statistically significant signs of toxicity observed in these short-term trials escalated to serious ill-health or not, EU regulators passed these products as substantially equivalent to non-GM corn and safe. If one is true to the science, these data suggest that approval of these GM corn varieties should be withdrawn until further long-term toxicity feeding studies are conducted because they are not substantially equivalent to non-GM corn and are potentially toxic.

Similarly, the Genetic Engineering Approval Committee, which is responsible for evaluating the safety of GM foods in India, ignored the worrying findings from the short-term feeding studies of Bt brinjal. Fortunately, the former Indian Minister for the Environment (Jairam Ramesh), responsible for overseeing the Bt brinjal application, did take note of the limitations of the safety tests available at the time as highlighted by scientists from around the world and sensibly did not approve this product for commercial use (see Jayaraman, 2009).

Studies conducted by academic researchers

Independent academic (university, institute)-based researchers have over the years found it very difficult to obtain GM crop material with which to conduct their own toxicity investigations. Nevertheless, following is a summary of studies with GM crops that have been completed:

- Rats fed GM Bt corn over three generations suffered damage (areas of necrosis) to liver and kidneys and alterations in blood biochemistry (Kilic & Akay, 2008).
- Old and young mice fed GM Bt corn MON810 showed a marked disturbance in immune system cells and in biochemical (cytokine) activity (Finamore et al., 2008).
- Rats fed GM Bt rice developed significant differences as compared with rats fed the non-GM isogenic line of rice. These included differences in the populations of

gut bacteria — the GM-fed group had 23% higher levels of coliform bacteria. There were differences in organ weights between the two groups, namely in the adrenals, testis and uterus. The authors concluded that the findings were most likely due to 'unintended changes introduced in the GM rice and not from toxicity of Bt toxin' in its natural, non-GM form (Schröder et al., 2007).

- Ewes and their lambs fed GM Bt corn variety Bt176 over three generations showed hyperplasia of ruminal epithelial basal cells in ewes and a disturbed gene functioning of liver and pancreas as revealed by smaller cell nuclei containing increased amounts of heterochromatin and perichromatin granules in lambs (Trabalza-Marinucci et al., 2008).
- A short-term (31-day) feeding trial in pigs with GM Bt corn variety MON810 showed significant differences in numerous immune cell type numbers (e.g. CD4+ T cells, B cells, macrophages) and biochemistry (cytokine levels; e.g. IL-12, IFN γ , IL-6, IL-4, IL-8) in the GM-fed group compared to the non-GM controls (Walsh et al., 2011). Despite the statistical significance of these differences the authors questioned the biological relevance of these observations, which is scientifically difficult to understand especially given the short duration of the investigation.
- Mice fed GM soy showed disturbed liver, pancreas and testes function. The researchers found abnormally formed cell nuclei and nucleoli in liver cells, which indicate increased metabolism and potentially altered patterns of gene expression (Malatesta et al., 2002; Malatesta et al., 2003; Vecchio et al., 2004).
- Mice fed GM soy over their lifetime (24 months) showed more acute signs of ageing in the liver than the control group fed non-GM soy (Malatesta et al., 2008).
- Rabbits fed GM soy showed enzyme function disturbances in kidney and heart (Tudisco et al., 2006).

Although narrower in scope than the industry-led studies in terms of parameters measured, these investigations showed consistent and significant signs of toxicity to multiple organ systems in response to the consumption of the GM feed.

Collectively, these industry- and academic-led feeding studies of commercialised GM soy and corn, which are already in the food and feed chain, found consistent signs of toxic effects in liver and kidney structure and function as well as some immune system disturbances. Such effects may be markers of the onset of chronic disease, requiring long-term rather than these reported short- and medium-term studies, to assess this more thoroughly. Unfortunately, such long-term feeding trials on GM foods are not required by regulators anywhere in the world (Séralini et al., 2011).

Mechanistic causes of negative health outcomes

What could be causing these worrying signs of toxicity in these animal feeding trials? At present we do not know. However, there are at least three logical mechanisms by which these GM crops can give rise to the disturbances in physiological and biochemical function and even signs of toxicity observed in these feeding studies:

- Bt toxin
- Herbicide residues
- Mutagenic effects of the GM transformation process

Effects arising from mutagenicity of GM transformation process

The GM transformation process (tissue culture plus GM transgene insertion) is highly mutagenic on two levels. Firstly, GM transgene insertion is random but with the transformation procedure ultimately selecting for insertion events within or near active plant host genes resulting in a high risk of host gene functional disruption by 'insertional mutagenesis'. The plant tissue culture component of the GM transformation process causes hundreds if not thousands of genome-wide

mutations (Latham et al., 2006; Wilson et al., 2006). Although any insertional mutagenesis effects are fixed, many of the genome-wide, tissue-culture-induced mutagenic events will be bred out of the plant during production of the commercialised GM crop. Many of the remaining mutagenic events will be benign but many run the risk of causing marked disturbances to host gene structure and function resulting in altered biochemistry and composition.

Many studies using the latest 'molecular profiling' technology have now been published which clearly demonstrate the impact on food crop composition resulting from the mutagenic effect of GM transformation. Listed below are some representative examples:

1. Studies of commercialised Bt corn variety MON810 have shown that this crop displays:
 - (a) A marked disturbance in protein composition profile specifically related to the GM transgene insertion event;
 - (b) A newly expressed protein: zein, a well-known allergenic protein;
 - (c) Differential response to environmental inputs as a result of the genome rearrangement derived from GM gene insertion;
 - (d) Truncation of seed storage proteins (Zolla et al., 2008);
 - (e) Disturbance in amino acid profiles (Manetti et al., 2006; Herrero et al., 2007);
2. Studies of non-commercialised GM rice have shown:
 - (f) GM rice engineered to be resistant to fungal diseases showed that not only were the structure of the seeds markedly altered in some cases but more importantly varied significantly in their composition compared to their non-GM counterparts (20 to 74% for amino acids; 19 to 38% for fatty acids; 25 to 57% for vitamins; 20 to 50% for elements; 25% for protein) (Jiao et al., 2010).

- (g) GM rice engineered with CryIAC Bt toxin and sck insecticide genes showed marked biochemical and nutritional disturbances; e.g., concentrations of glycerol-3-phosphate, citric acid, oleic acid and sucrose increased considerably (Zhou et al., 2009).

These studies show that at the very least, when analysed properly in detail, no GM crop can be classified as substantially equivalent to its non-GM counterpart and on this basis passed as safe. Disturbances in plant biochemistry can result in novel toxin production, and may account at least in part for the signs of toxicity observed in animal feeding studies.

Bt toxin

Bt toxin is a crystalline protein complex that occurs naturally in the common soil bacterium *Bacillus thuringiensis*. Some types of Bt toxins are effective insecticides and have been used in agricultural spray form for many years by both conventional and organic farmers alike. However, Bt toxin in its native crystalline form is inactive as an insecticide. In the digestive tract of certain insects it is broken down to release the subcomponent ('Cry protein') that is active as an insecticide. This activation procedure makes Bt toxin a highly selective insecticide as only certain insects possess the appropriate acidic conditions in their digestive tracts to bring about this conversion. Once activated, the Bt toxin inserts into and causes lesions in the insect's gut epithelium bringing about death either through a disrupted digestion or systemic bacterial infection (Vachon et al., 2012).

How does native Bt toxin used as an agricultural spray compare with Bt toxin engineered into GM crops? It is important to note that Bt toxins engineered into all GM crops consist only of the active component. As a result, the GM crop contains throughout its structure high levels of constitutively active Bt toxin that is as a result approximately only 45% identical to the native form. This makes the Bt toxin in GM crops significantly different

from that used as an agricultural spray; its insect target specificity is compromised (e.g. see Schmidt et al., 2009) and it may pose new health risks.

Why is Bt toxin a health concern?

Bt toxin has been proven to be an allergen and potent adjuvant in mammals even at low levels of exposure (Vázquez et al., 1999; Vázquez-Padrón et al., 1999 & 2000; Kroghsbo et al., 2008; Adel-Patient et al., 2011). That is, the organism can readily mount a cellular and humoral immune response against Bt toxin and that Bt toxin can markedly augment immune responses against other ingested foodstuffs. The adjuvant properties of Bt toxin have been observed in sheep as well as rodent model systems where immune response to *Salmonella abortus ovis* vaccination was more efficient in GM-corn-fed sheep than non-GM-fed controls (Trabalza-Marinucci et al., 2008). Therefore, Bt toxin possesses properties which, with sufficient exposure, could lead to allergic reactions caused directly by itself or against other ingested foodstuffs. These properties may account for the disturbing effects on immune system function observed in animal feeding studies detailed above (Finamore et al., 2008; Walsh et al., 2011). In addition, they may account for the well-documented but poorly officially investigated incidences of allergic reactions in the human population linked to exposure to GM Bt toxin-containing crops and foods. Accidental entry into human foods of GM Cry9C Bt toxin 'Starlink' corn intended only for animal feed, led to many instances of allergic-type reactions following consumption of contaminated food (CDC, National Center for Environmental Health, 2001). Workers harvesting cotton in Bt cotton fields in India suffered severe skin rashes and in some cases needed hospitalisation (Gupta et al., 2005) with farm animals feeding on the Bt cotton stubble suffering severe illness and death (Warangal District, Andhra Pradesh, 2006).

A recent finding is that Bt toxin type Cry1Ab, which is present in commercialised GM crops such as MON810 corn, binds to human cells in

tissue culture, causes disturbances in energy production and exterior (plasma) membrane systems leading to cell death, albeit at relatively high levels (Mesnage et al., 2012).

Furthermore, a study conducted on pregnant and non-pregnant women in Canada found Bt toxin protein circulating in the blood of pregnant women and the blood supply to their foetuses, as well as in the blood of non-pregnant women (Aris and Leblanc, 2011). Although the source of the Bt toxin detected in these people is unknown, this study shows that Bt toxin can survive digestion and enter the circulation. This raises the possibility that people who consume Bt GM crops in moderate to large quantities as a staple food run the risk of chronic systemic exposure to this insecticide, which, based on the outcomes from animal feeding studies, may contribute to adverse health effects especially with respect to liver, kidney and immune system function. Therefore, further investigation is needed before Bt crops can be claimed to be safe for humans.

Conclusions

An increasing body of evidence shows the disruptive effect of the GM transformation process and clear signs of toxicity in well-controlled animal feeding studies even of a short-term nature. These observations demand that toxicity be confirmed or refuted in life-long animal feeding studies. In studies with Bt toxin GM crops that have shown signs of toxicity it is not possible at present to distinguish whether the cause is either the Bt toxin or the mutagenic effect of the GM transformation process or a combination of both. Future studies need to address this point by including a control of non-GM feed with added Bt toxin preferably from a GM plant source compared to GM and non-GM feed alone. Allergenicity needs to be evaluated with human volunteers since there are no animal model systems available for this type of clinical investigation.

Based on available evidence and inadequacy of the tests required by regulators, at present no GM crop and food can be categorically

stated as safe to consume, especially on a long-term, life-long basis.

Dr Michael Antoniou is research group leader and lecturer in molecular genetics, Department of Medical and Molecular Genetics, Kings College London School of Medicine, UK. He has 32 years of experience in the use of genetic engineering technology investigating gene organisation and control with over 50 peer-reviewed publications of original work and holds inventor status on a number of gene expression biotechnology patents. Current research interests are to exploit his discoveries in gene control mechanisms for the production of research, diagnostic and therapeutic products and safe and efficacious human somatic gene therapy for inherited and acquired genetic disorders. Dr Antoniou has acted as an adviser on biotechnology issues to many organisations and NGOs both within the UK and abroad, has sat on the UK government's GM Science Review Panel for the second of its 'GM Nation?' public debates, has advised political parties on applications for GM in medicine and agriculture, and has been an active contributor internationally to media outlets, public meetings and conferences.

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